ANNUAL REPORT OF PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1972
PART I

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

U.S. NATIONAL CANCER INSTITUTE

Fiscal Year 1972

Part I

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1971 through June 30, 1972

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ANNUAL REPORT
NATIONAL CANCER INSTITUTE
July 1, 1971 - June 30, 1972

Introduction

Fiscal Year 1972 was marked by a number of unprecedented developments affecting the National Cancer Institute, highlighted by: 1) the largest monetary operating level in the Institute's history; 2) the signing into law of The National Cancer Act of 1971, bringing a variety of changes in the Institute's operations; 3) the acquisition by NCI of laboratory facilities at the Army's former Biological Defense Research Center at Fort Detrick, Maryland; and 4) the appointment by the President of a new Institute Director.

For the year covered by this report, the President approved the appropriation by the Congress of $237,531,000 for the NCI, and at his urgent request the Congress voted an extra $100 million supplement to the 1971 appropriation, to give the Institute an operating level in 1972 of $337,531,000, an increase of $107,148,000 over the 1971 appropriation. (The extra $100 million was made available for the Institute's use until June 30, 1973. In May 1972 the President sent to the Congress a request for a $40 million supplement to the FY 1972 NCI appropriation - $36 million of it for construction grants and the balance for fellowship and training grants.

In January 1972 the President's fiscal year 1973 budget request to the Congress for the National Cancer Institute was $430 million. Senate-House Appropriations Conference Committee could raise this figure before final passage of the bill, however.

On December 23, 1971 the President signed into law The National Cancer Act of 1971, authorizing an appropriation of $1.6 billion for cancer research over the ensuing three years and directing the President to seek additional funds from the Congress as necessary to pursue immediately any development in the national cancer program for which appropriated funds are not available.

The Act provides increased authorities and responsibilities for the Director of the National Cancer Institute, who will report directly to the President on matters concerning the budget of the National Cancer Institute.

The Act also provides a 3-member President's Cancer Panel, appointed by the President to monitor the national cancer program and submit directly to him periodic progress reports on the program, and annually an evaluation of the efficacy of the program and suggestions for improvements, and shall submit such other reports as the President may direct.

The Act authorizes the Director of the NCI, which will be located within the National Institutes of Health, to:

- Plan and develop an expanded, intensified, and coordinated cancer research program encompassing the programs of the Institute, related programs of the other research institutes, and other Federal and non-Federal programs, with the advice of the National Cancer Advisory Board.

- Utilize existing research facilities and personnel of NIH for accelerated exploration of opportunities in areas of special promise.

- Encourage and coordinate cancer research by industrial concerns which evidence a capability for such research.

- Collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer, including the establishment of an international cancer data bank.

The Act also authorizes the establishment of 15 new centers for clinical research, training, and demonstration of advanced diagnostic and treatment methods related to cancer. For the planning, establishment, strengthening, and basic operating support of existing or new centers, cooperative agreements with public and private nonprofit agencies are also authorized.

Cancer control programs shall be established as necessary for cooperation with State and other health agencies in the diagnosis, prevention, and treatment of cancer.

Additional provisions of the Act include:

a. The Directors of the National Institutes of Health and of the National Cancer Institute are to be appointed by the President.

b. The President will receive from the Director of the National Cancer Institute a scientific review of the program each year and a plan for the program during the next five years.

c. The President will carry out and submit to Congress an administrative review within 1 year of the legislation's enactment with recommendations for any needed legislative changes.

d. The budget of the National Cancer Institute will go directly from the Director of the National Cancer Institute to the President with opportunity for comment, but not change, by the Secretary of the Department of Health, Education, and Welfare, the Director of the National Institutes of Health, and the National Cancer Advisory Board. Appropriated funds will go directly from the President and Office of Management and Budget to the National Cancer Institute.

e. The President is authorized to seek additional funds for the cancer effort from the Congress if necessary to pursue immediately any development in the national cancer program.

f. The National Cancer Advisory Board will be composed of 23 members--18 appointed by the President--not more than 12 of whom may be scientists and

physicians among the leading authorities in the cancer field. Also on the Board will be five Federal officials who shall serve ex officio.

g. The President's Cancer Panel will make periodic progress reports directly to the President. At least two members must be distinguished scientists or physicians. It will meet at the call of the Chairman but not less than 12 times a year.

At the time that he signed this legislation, on December 23, 1971, President Nixon announced the appointment of Benno C. Schmidt, Managing Partner of J. H. Whitney and Company, New York City, and former Chairman of the National Panel of Consultants on the Conquest of Cancer, as Chairman of the President's Cancer Panel. On January 31, 1972, the appointments of Dr. Robert A. Good, Professor of Pediatrics at the University of Minnesota Medical School, Minneapolis, and Dr. R. Lee Clark, President of the University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, as members of the Panel were announced.

The eighteen members of the National Cancer Advisory Board were designated by the President on March 7, 1972. At the same time it was announced that members of the National Advisory Cancer Council, which was superseded by the Board, would continue to serve on the Board until such time as their Council memberships shall have expired.

The appointment of Dr. Frank J. Rauscher, Jr., Scientific Director for Etiology, NCI, to the post of Director of the Institute was announced by the President on May 5, 1972. Dr. Rauscher, who succeeded Dr. Carl G. Baker as NCI Director, is an eminent virologist with the Ph.D. degree in Microbiology from Rutgers University, where he taught and conducted research for four years before joining NCI in 1959 as a microbiologist in the Laboratory of Viral Oncology.

During the winter and spring of 1971-72 approximately 250 biomedical scientists with expertise in laboratory and clinical research participated in a series of meetings at the Airlie Conference Center near Warrenton, Virginia, to develop the National Cancer Program Plan. Organized into 41 interdisciplinary panels, the experts recommended scientific approaches to seven key objectives, providing the basis for the programs to be implemented under the expanding NCI cancer research effort. In addition to their scientific advice, they provided guidance in the organization and management of the national research program. The recommendations emerging from the meetings will be translated into action programs in fiscal 1973.

Goals of the National Cancer Program Plan are to significantly reduce 1) the number of new cancer cases occurring in a given period; 2) the ratio of cancer patients to the well population; and 3) the ratio of cancer deaths to the total number of cancer cases. These goals will be achieved through research in prevention (the nature and causation of cancer), detection and diagnosis, treatment, and rehabilitation.

The Plan's seven objectives were defined early in 1971:

1. To reduce the effectiveness of external agents in increasing the probabilities of development of cancers in existing individuals or in individuals of subsequent generations.

2. To modify individuals (e.g., by vaccination) to decrease the likelihood of cancer development, both in the current generation and in subsequent offspring.

3. To prevent conversions of cells to those capable of forming cancers (i.e., block, or interfere with the proximate step, or steps, involved in conversion to cells capable of forming cancers).

4. To prevent tumor establishment from cells already capable of forming cancers (e.g., transformed cells, cells constituting precancerous tissues; etc.).

5. To achieve an accurate assessment of the presence, extent and probable course of cancer risks in population groups (including attention to precancerous lesions) and of cancers in individuals alone (diagnosis) and in groups (detection) as an aid to prevention, cure, or prognosis.

6. To cure as many patients as possible, and to maintain maximum control of the cancerous process in patients not cured.

7. To restore patients with residual deficits as a consequence of their disease or treatment to as nearly a normal functioning state as possible.

On October 18, 1971, President Nixon announced that the Army's Biological Defense Research Center at Fort Detrick, Md., would be converted into a leading center for cancer research as part of the major campaign to conquer cancer. Laboratories and animal facilities valued at more than $70 million were turned over to the Institute. Under the new program the facilities will be dedicated to research on the causes and treatment of cancer, the nation's second major cause of death. By July 1972, or soon thereafter, cancer research will be underway in some of the laboratories; within the next 3-5 years an estimated 80 percent of the laboratories could be operational.

The cancer research facilities at Fort Detrick will be managed by a private contractor, and the operation will be the Institute's largest single contract. An initial contract figure of two to three million dollars this fiscal year is expected to increase to $15 to $20 million in five years.

Virus-Cancer Research

The foundations for much of the progress made during the reporting year in research on cancer viruses were the separate discoveries announced in June 1970 of the enzyme, RNA-dependent DNA polymerase, also called "reverse transcriptase," by Drs. Howard Temin (McArdle Laboratories, University of Wisconsin)

and David Baltimore (Massachusetts Institute of Technology). Until discovery of this enzyme, RNA, or ribonucleic acid, was considered to act in cells only as a servant chemical that usually was formed from a blueprint of DNA, or deoxyribonucleic acid, to carry out DNA's instruction in the cell. The new finding demonstrated that this enzyme enables RNA to be the blueprint for formation of DNA and thus control the cell's heredity. Discovery of reverse transcriptase enabled scientists to understand how certain animal viruses, whose cores contain RNA instead of DNA, can alter a cell's genetic material and consequently cause certain types of cancer in animals.

Candidate Human Viruses

Because type-C RNA viruses have been strongly implicated in certain human cancers, scientists have worked to isolate such viruses from human tissues. Thanks to the discovery of reverse transcriptase, new techniques are now available to detect viral activity in human cancer tissue. These advances in study of cancer viruses have contributed to the biochemical evaluation of three candidate human cancer viruses discovered this year.

In the July 14, 1971 issue of Nature New Biology Drs. Elizabeth S. Priori, Leon Dmochowski, Brooks Myers and J. R. Wilbur of M.D. Anderson Hospital and Tumor Institute, Houston, Texas, under contract to the NCI Special Virus Cancer Program (SVCP), reported isolation of a type-C virus, named ESP-1, in cultured cells from a five-year-old American child with Burkitt's lymphoma, a relatively rare lymphatic cancer which usually affects the jaw and facial area of young African children.

First immunological studies of this virus by the M.D. Anderson group indicated that it did not cross-react with any known animal RNA type-C cancer virus and thus might be a human virus. Subsequent studies such as those reported in the September 10 issue of Nature by NCI scientists Drs. Wade P. Parks, Robert J. Huebner, and George J. Todaro, and others receiving support from the SVCP, indicated that the virus showed similarity to the leukemia virus of the mouse. Conversely, in November 1971, at the Symposium on Mammary Neoplasia in Cherry Hill, New Jersey, Dr. Albert J. Dalton of NCI described what he believes to be structural differences between ESP-1 and every known subtype of mouse leukemia virus as viewed under the electron microscope.

Studies are now underway to determine whether the observed cross-reactivity between the mouse virus and ESP-1 virus may be due to an immunologic phenomenon common to the two species. Such a finding would indicate that the ESP-1 virus is at least unique and could be of human origin.

Early in December 1971, scientific teams working independently in the Washington, D. C. area and in Los Angeles, both cooperating in the NCI Special Virus Cancer Program, also reported what they believe to be new candidate human cancer viruses.

The discoveries were announced at the Children's Hospital of Los Angeles by Drs. Robert M. McAllister and Murray B. Gardner of the University of

Southern California's School of Medicine and Dr. Robert J. Huebner, Chief of the Viral Carcinogenesis Branch, NCI, and in Washington, D. C., by Drs. Sarah Stewart and William Feller of Georgetown University. Both groups found the viruses, which may be the same, and are presumed to be cancer-causing in cells from cancers of skeletal muscle called rhabdomyosarcoma. The virus found by the USC group has been studied by several scientific teams within and supported by the NCI and found to be immunologically unique. It does not cross-react with any of the known animal cancer viruses. Scientists at Georgetown University now are evaluating their virus with similar tests. Further efforts are in progress, or planned, to determine whether these viruses are indeed of human origin, whether they occur primarily in human cells, and whether they are capable of causing cancer in animals.

Viruses and Breast Cancer

In the May 14, 1971 issue of Nature, Drs. Jeffrey Schlom and Sol Spiegelman of the Institute of Cancer Research, Columbia University, and Dr. Moore reported reverse transcriptase activity in human milk samples which contained virus particles. In November 1971, at the Symposium on Mammary Neoplasia, Dr. Spiegelman reported that his group has been able to use a hybrid molecule, consisting of DNA from the mouse breast cancer virus and human RNA, as a probe for virus-specific information in human milk samples.

Also in November 1971 these scientists announced in the journal Science the development of a special test for simultaneous detection of reverse transcriptase and the high molecular weight RNA unique to RNA cancer-causing viruses. Biochemical detection of such cancer virus "footprints" in human tissue previously has been hampered by the low concentration of virus activity as well as by the presence of cell debris in the human material. Drs. Schlom and Spiegelman consider their test sufficiently simple, sensitive, and discriminating to signal the presence of a virus or viral components in biological fluids such as human milk. They devised their test with the mouse breast cancer virus and also have tested cat and chicken cancer viruses with the method.

Biochemistry of RNA Cancer Viruses

A number of investigators have shown the presence of reverse transcriptase enzymes in all type-C RNA cancer viruses. In the May 1971 issue of the Proceedings of the National Academy of Sciences, Drs. Stuart Aaronson, Wade Parks, Edward Scolnick and George Todaro of NCI showed that in the various mammalian viruses these enzymes are immunologically related. These investigators and others are now exploring ways of inhibiting the activity of the enzyme in the virus by immunologic and chemical means as a potential approach toward controlling cancer.

In September at the Vth International Symposium on Comparative Leukemia Research in Padua, Italy, sponsored jointly by NCI and the Leukemia Society of America, Inc., Dr. Raymond Gilden announced that he, Dr. Stephen Oroszlan, and Mr. David Bova of Flow Laboratories, Inc., Rockville, Maryland had

developed antibody to the leukemia viruses of four different species of animals. The antibody developed by these scientists is now used extensively to analyze new mammalian cancer viruses that are isolated, including candidate human cancer viruses.

DNA Viruses Associated with Cancer of the Uterine Cervix

DNA viruses also have attracted much attention during the past year. In November, 1971, a team of NCI-supported scientists at the Johns Hopkins University, Baltimore, Maryland, led by Dr. Laure Aurelian, isolated herpesvirus type-2 (HSV2) from cervical cancer cells of a patient by growing them in highly alkaline broth in laboratory test tubes. Dr. Aurelian's work is the latest in a long chain of studies indicating that the relationship between HSV2 and cervical cancer is more than coincidental.

Viruses and Hodgkin's Disease

In the September 10 issue of Nature, scientists at the Sloan-Kettering Institute for Cancer Research, New York City, working with partial support from NCI, reported association of viruses with another type of human cancer, Hodgkin's disease. Drs. Magdalena Eisinger, F. Kingsley Sanders and Associates were able to grow cells from patients with Hodgkin's disease which contained evidence of both RNA viruses and DNA viruses, the latter tentatively identified as herpes-type. These findings, while they are not evidence that these viruses cause Hodgkin's disease, are significant because they suggest the interaction of two types of viruses in the cause and course of the disease.

Tumor-causing DNA viruses

In a project to learn about the lipid composition of cells, NINDS scientists, in collaboration with the NCI, have detected a definite chemical change on the surface of DNA virus-induced cancer cells.

Using tumor-causing DNA viruses to transform normal cells into cancer cells, the scientists found that there is a block in the synthesis of complex lipids on the surface of the cancer cells. They further identified the site of this enzymatic block in the synthesis of the complex lipids called gangliosides.

The investigators report that when tumor-causing DNA virus is incorporated into the genetic apparatus of a mouse cell, the ganglioside pattern on the cell membrane is changed. The gangliosides become more simple and do not produce the usual patterns of a normal cell because of the enzyme block.

This is the first time a defined chemical change has been identified on the surface of simian (monkey) virus 40 and polyoma (tumor) virus-transformed cells. The researchers hope to convert the cancer cell back to a normal cell by overcoming the genetic block.

These findings were reported by Drs. Roscoe O. Brady and Edwin H. Kolodny and Frederico A. Cumar in the NINDS Laboratory of Neurochemistry and Dr. Peter T. Mora and Vivian W. McFarland in the Macromolecular Biology Section, Laboratory of Cell Biology, NCI.

Genes and Cancer

During 1971 many Special Virus Cancer Program scientists reported results of experiments to understand the role of genetics in cancer. For example, at the NCI-supported Vth International Symposium on Comparative Leukemia Research in Padua, Italy in September, Dr. Hans Meier reported that he and Drs. B. Taylor and D. Myers of the Jackson Laboratory, Maine, had determined which gene controls gs-antigen expression in animal RNA cancer viruses. Antibody, protein that reacts specifically with this type of antigen, has proven invaluable in immunologic studies to identify animal cancer viruses.

Effect of Chemicals on Cancer

In cancer virology, laboratory tools and models are necessities; hypotheses in this area can rarely be tested in man. Last year, NIAID scientists developed an important technique for leukemia research and reported results lending powerful support to the "oncogene" theory of cancer causation espoused by a number of NIH scientists--that instructions for a leukemia virus may be present in unexpressed form in the DNA, or blueprint material, of seemingly normal cells. Using either of two chemicals--5-iododeoxyuridine (IUDR) or 5-bromo-deoxyuridine (BUDR)--Dr. Wallace P. Rowe and his associates activated mouse leukemia virus in mouse cell lines where none was formerly present. In fact, by means of these chemicals, it was shown that every cell in the cultures had the capacity to produce virus.

Extending this work, Drs. Stuart Aaronson, George Todaro, and Edward Scolnick of NCI showed by a similar experiment that each genetically identical cell of a mouse embryo cell line could be stimulated to demonstrate the presence of virus.

Several other NCI-supported scientists have also demonstrated ability to trigger chemically the formation of a type-C RNA cancer virus in animal cells. In March 1971, at the 24th Annual Symposium on Fundamental Cancer Research, Houston, Texas, Drs. Robert J. Huebner and Gary J. Kelloff, NCI, and several groups of NCI contractors reported results of experiments to develop ways to make quick and precise identification of carcinogens, or cancer-causing chemicals, in the environment. One such study, by Dr. Aaron E. Freeman of Microbiological Associates in Bethesda, Maryland, showed in test tube studies that smog residues can trigger transformation, or cancerous changes, in animal cells chronically infected with various animal leukemia viruses. When the same concentration of smog residue or virus was used alone, transformation did not occur, suggesting the need for both the chemical and the virus interaction to produce the cancerous changes. Other scientists in the study found similar transformation of laboratory-grown cells for five other chemical carcinogens.

This work, coordinated by the Viral Carcinogenesis Branch of the NCI, could lead to the development of highly sensitive test tube assays of suspected carcinogens.

A New Model For Carcinogenesis and Teratogenesis

An experimental animal capable of developing in the laboratory the special forms of cancer common to childhood would be of considerable value as a tool in research into this leading cause of death in children. The American marsupial, the opossum, is of interest in this regard because its young are born in a fetal state (12-3/4 days gestation). This permits observation of the development of the young during the time when a mammalian fetus is *in utero*.

A continuing study of the response of the opossum exposed by the mouth at several stages of development from birth to three months to a chemical carcinogen (any cancer-producing substance) indicates that this animal may have a unique susceptibility to tumor formation. These experiments have already shown that tumors rarely produced in animals in the laboratory have been produced in the opossum. They originated early in the development of liver, kidney, lung, eye, and jaw of the opossum. Several forms of skin cancer have also developed in these animals. In addition birth defects in the form of kidney cysts are quite common in the animals exposed to the carcinogen, suggesting that the opossum may also be useful in studying the relationship between malformations and cancer.

The apparent lack of immunologic competence at birth makes the animal suitable for experimentation regarding the induction of tumors with human cancer viruses and for the transplantation of human tumors.

Third National Cancer Survey

In September 1971 the NCI published a preliminary report on the Third National Cancer Survey, analyzing cancer statistics in eight large metropolitan areas and one entire state. This report gives the cancer incidence for 1969 and indicates the following trends: (1) The overall incidence of cancer in men is increasing, a trend particularly marked among blacks, while in women it is decreasing. (2) The incidence of lung cancer has doubled since 1947 in both men and women of both races. (3) The incidence of cancer in blacks is substantially higher than in whites, a difference particularly large between black and white men. It is not likely that these differences in cancer incidence arise from genetic causes, but they are more likely to be due to socio-economic factors.

Data for the survey were collected in Iowa, Detroit, Minneapolis-St. Paul, Pittsburgh, Atlanta, Birmingham, Dallas-Fort Worth, Denver, and San Francisco-Oakland, with a combined population of over 20 million residents. A total of 61,409 new cancers were diagnosed in this population during 1969. Statistics generalized for the entire U. S. population indicate that, in addition to minor skin malignancies, 610,000 new cases of cancer were diagnosed that year.

The Third National Cancer Survey, a sequel to the Ten Cities Surveys of 1937 and 1947, is being conducted by the NCI Biometry Branch in conjunction with medical schools and associations, health departments, voluntary health agencies, and other organizations. In addition to data on cancer incidence, these groups are collecting information on the social and economic effects of cancer and the medical care needed by cancer patients. Preliminary attempts at evaluating the data have only begun. The final report, which will include data for the years 1969 through 1971, is due in 1973.

Chemicals and Cancer

With increasing chemical pollution of the environment, concern about carcinogens, or cancer-causing chemicals, has been growing. This concern was reflected in some of the important NCI activities and research during 1971.

Evaluating Carcinogenic Hazards

Staffs of the Food and Drug Administration and NCI Carcinogenesis area held a joint conference on the Criteria for Evaluation of Carcinogenic Hazards of Drugs in December 1971. The conference was coordinated by Dr. W. B. D'Aguanno of the FDA and Dr. Umberto Saffiotti, NCI's Associate Scientific Director for Carcinogenesis. Participants included representatives of the Pharmaceutical Manufacturers Association, the President's Science Advisory Committee, the National Academy of Sciences and several other scientific organizations. Plans were proposed for increased cooperation between the FDA and the NCI in monitoring hazardous drugs and food constituents.

The first annual meeting of the Lung Cancer Segment of the Lung Cancer Program in Carcinogenesis was held November 3-5, 1971. The Program supports studies in the causes and biological mechanisms of lung cancer as a basis for preventing this disease, which kills 68,800 Americans each year. The participants, NCI scientists and those under contract to the NCI, spoke on multiple factors in respiratory carcinogenesis and cellular and biochemical studies.

As part of a continuing effort to evaluate chemicals in the environment, the NCI awarded a contract in June 1971 to the Stanford Research Institute (SRI) to study in animals the effect of environmental chemicals in combination with other chemicals. As a result of another study, initiated in November 1970 by the NCI at the SRI, investigators announced in December 1971 the feasibility of a new information system called CHRIS (Cancer Hazard Ranking and Information System) to rank chemicals according to their relative cancer-causing potential for man. To date, the SRI scientists have used the system to assess a group of air and water pollutants, soaps and detergents, food chemicals, and agricultural chemicals. It can continually re-evaluate the ranking of hazardous chemicals as more data are added.

A fourth volume in the series "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" became available in early December 1971. The new publication, prepared under an NCI contract by John I. Thompson and Company,

Rockville, Maryland, includes reports for the years 1968 and 1969. The 650-page volume continues work formerly undertaken by Drs. Jonathan L. Hartwell and Philippe Shubik in their first three surveys of the scientific literature through 1960. NCI plans at least two additional supplements, 1961-1964 and 1965-1967.

N-nitrosamines in Cigarette Smoke Condensate

NCI-supported investigators, Drs. Donald Johnson and John W. Rhoades of the Southwest Research Institute, San Antonio, Texas, announced in December 1971 that they had identified a type of cancer-causing chemical called N-nitrosamines in cigarette smoke condensate. These chemicals have long been considered as possible components of tobacco smoke condensate, but the investigators are the first to demonstrate their presence. The scientists also reported that the amount of N-nitrosamine in cigarettes depends on the variety of tobacco used and the amount of nitrogen in the soil in which the tobaccos were grown. This study is part of an effort to complete the profile of chemical constituents of cigarette smoke condensate, so that cancer-inducing substances can be identified and eliminated.

Occupational Hazards

A study reported in the April 16, 1971 issue of Nature by Drs. E. K. Weisburger, J. M. Rice, and J. H. Weisburger indicates the carcinogenicity in rats of two industrial chemicals, propylene imine and propane sultone. Propylene imine, an important chemical intermediate with many applications in the production of polymers, coatings, adhesives, textiles and paper finishes, caused tumors in a wide range of organs in the test animals. Propane sultone, a chemical with many potential uses in the detergent, textile, rubber, pharmaceutical and agricultural chemical industries, was equally carcinogenic. The investigators emphasized the need for adequate precautions in handling these compounds because of their potential deleterious effects in living systems.

Another widely used industrial chemical, bis (chloromethyl) ether, was also identified as a potential occupational hazard. In a preliminary report in the Archives of Environmental Health in August 1971, Prof. Sidney Laskin, an NCI contractor at the New York University, and his co-workers described the development of tumors of the lung and nasal cavity in rats that inhaled bis (chloromethyl) ether at extremely low concentrations.

Cancer Detection

In 1971 steps were taken to further the development of tests for the detection of some of the major forms of cancer in early, presymptomatic stages. In July, funding of an NCI contract to evaluate an early detection test for cancers of the colon and rectum was announced. Contract investigators at Tufts University School of Medicine in Boston, Dr. Robert Schwartz and his colleagues, are examining the specificity of a test, developed by Dr. Phil Gold

of Canada, which measures carcinoembryonic antigen (CEA), a substance which has been found in the blood of patients with these cancers and may be a sign of very early cancer.

A project to improve survival of lung cancer patients was initiated at the Mayo Clinic, Rochester, Minnesota, under an NCI contract. Dr. Robert S. Fontana and his co-workers are screening men at high risk of developing lung cancer due to smoking. They are using chest X-rays and microscopic examination of cells obtained from deep cough sputum, the only procedures so far proved useful in detecting presymptomatic lung cancer. Although the techniques used are not new, this will be the first large-scale surveillance of persons at high risk to lung cancer studied with these techniques. The investigation will also evaluate other early detection methods and procedures for locating tumors too small to be seen on an X-ray.

Preliminary findings from an NCI contract-supported study on the value of a breast cancer detection method were published in the March 15, 1971 issue of the Journal of the American Medical Association. The investigators, Mr. Sam Shapiro, Dr. Phillip Strax, and Dr. Louis Venet, based their report on data from 62,000 women between the ages of 40 and 64 enrolled in the Hospital Insurance Plan of Greater New York. Half the group was offered four annual breast examinations with mammography (a special X-ray technique) and regular clinical procedures. The other half followed their usual practices in receiving medical care and did not participate in the planned annual screening program. Results indicate 40 percent fewer deaths due to breast cancer among women screened with mammography and clinical examination, compared with breast cancer deaths occurring among the unscreened group. Based on this preliminary report on the effectiveness of such a screening program, the investigators recommended modifications in mammography and training of more medical personnel in order to screen large portions of the population for breast cancer.

CANCER DRUGS

During the reporting year, research on cancer chemotherapy, the drug treatment of cancer, continued to be an important part of NCI's effort to control cancer. Research efforts ranged from the search for potential new drugs to finding the most effective means of using the drugs now available.

Development of New Cancer Drugs

A total of 14,226 new compounds was tested by NCI in calendar 1971 as potential anti-cancer agents. These included approximately 9,000 synthetic chemicals and 5,000 natural products from fermentation, plant, and animal sources.

The largest numbers of tests were performed using mice with leukemia L-1210, a tumor model found to be the most reliable system for predicting the response of human cancer to drug therapy. The number of tests with these mice increased 17 percent in 1971 compared to 1970.

The next most frequently used tumor model was the P-388 mouse leukemia, similar to the L-1210 but able to detect the activity of smaller amounts of crude material. It was therefore used to test some of the natural products. The total number of tests in 1971 with the various tumor models and cell systems was 13 percent higher than in 1970.

As a result of such initial screening against rodent cancers, nine materials showed sufficient anti-tumor activity to be selected by NCI scientists for pharmacology-toxicology studies in large animals. Such studies are prerequisite to drug evaluations in human cancer, in order to assure that the test compounds will be relatively non-toxic and safe to use. Moreover, they help to determine the starting dose for human drug administration, which usually begins in NCI-sponsored drug evaluations at one-tenth the dose tolerated by dog or monkey (whichever is less).

During calendar year 1971 seven compounds passed the NCI's stringent tests in animals for pharmacologic evidence of activity and safety, and were accepted for study against human cancer. The materials selected for these important tests are methyl-CCNU; cis-diamminedichloro-platinum; ICRF-159; and 9-B-D-arabinofuranosyl- 9H-purine-6-thiol; chromomycin A3; estriol; and gallium nitrate. During 1971 the National Cancer Institute filed Investigational New Drug applications with the Food and Drug Administration to conduct clinical studies of the first four of these drugs plus 1-propanol,3,3'-iminodi,dimethanesulfonate hydrochloride; 5-azacytidine; iphosphamide; and rifamycin SV. Then the painstaking, long-term clinical studies were begun to determine whether or not the materials will offer cancer patients therapeutic benefits not now available through conventional methods of treatment.

Clinical Evaluation of New Cancer Drugs

Promising results on two relatively new anticancer drugs were reported during the year. Adriamycin, an antibiotic developed by the Farmitalia Research Laboratory, an Italian firm, has been found effective against a wide range of human cancer, including some of the solid tumors seldom responsive to drugs. Two groups of NCI grantees, participants in the Acute Leukemia Cooperative Group B and the Southwest Cancer Chemotherapy Study Group, reported results with this drug in the October 1971 issue of Cancer. They studied a total of 153 patients with advanced cancer, some of whom had received prior chemotherapy. Among the patients responding to the drug were those with acute lymphocytic leukemia, acute myeloblastic leukemia, neuroblastoma, Wilms' tumor, reticulum cell sarcoma, Ewing's sarcoma, osteogenic sarcoma and cancers of the lung, bladder, and breast. The patients who responded experienced either a reduction in the size of their tumor or complete, though temporary, disappearance of their cancer. Further study will determine the most effective dosage and schedule for adriamycin and the cancers most responsive to this drug.

The second promising drug, trimethylcolchicinic acid (TMCA), a compound that inhibits cell division, was one of a group of drugs tested in a study of 73 patients with advanced malignant melanoma, a usually fatal cancer that may develop from a pre-existing mole. NCI grantees, Drs. F. Deborah Johnson and

Edwin M. Jacobs of the Cancer Research Institute, San Francisco, reported their 10-year study (January, 1960--December, 1969) in the June, 1971 issue of Cancer. TMCA produced significant responses comparable or superior to any other drug or combination of drugs used. In addition, TMCA was easy to administer and was tolerated reasonably well by a majority of patients.

Treatment with Combinations of Drugs

With the use of combinations of anti-cancer drugs, long-term survival has been achieved in patients with advanced stages of Hodgkin's disease, a cancer of the lymph system. NCI scientists Drs. George Canellos, Robert C. Young, and Vincent T. DeVita discussed the survival of a group of patients treated at the Institute between 1964 and 1967 at the Hodgkin's Disease Symposium held in October 1971 in St. Louis, Missouri under the sponsorship of the Cancer Clinical Investigation Review Committee and the NCI Clinical Investigations Branch. (Advanced Hodgkin's disease is treated with drug therapy; early-stages of the disease are treated by radiation, as discussed below.)

Of the 43 patients in the study group, 35 achieved complete remission, that is, apparent disappearance of their disease. Of these 35 patients, 24 are now alive without evidence of cancer 4 to 7 years after the start of treatment with a combination of vincristine, procarbazine, prednisone, and an alkylating agent. Fifteen of these 24 patients have remained in complete remission with no need for further treatment after initial chemotherapy. Since these 24 patients represent 69 percent of the original 35 patients who achieved complete remission of their disease, complete response to initial combination therapy may forecast long-term survival.

Combination chemotherapy initiated in 1967 at the NCI for patients with advanced lymphosarcoma, another type of cancer of the lymph system, is achieving higher survival rates than treatment programs previously reported. Dr. Charles M. Bagley and his co-workers discussed the use of cyclophosphamide, vincristine, and prednisone in combination at the annual meeting of the American Association for Cancer Research in Chicago in April 1971. Of the 35 patients in the study, 20 (57 percent) had complete remissions of lymphosarcoma, 12 (34 percent) had partial remissions, and 3 (9 percent) failed to show any response. Of the 20 patients who achieved complete remissions, 18 had remained in a disease-free state for 1 year at the time of reporting. In addition, 28 patients (79 percent) were alive 1 year after initial treatment and 26 patients (73 percent) remained alive 2 years after treatment.

Treatment of African Lymphoma

Cyclophosphamide is a drug known to be effective against Burkitt's lymphoma, a cancer of the lymph system which has been studied primarily among African children. Study of the drug over the past four years at the Lymphoma Center in Uganda, where the disease appears to be endemic, has revealed that the outlook for survival and recovery from this disease is independent of initial treatment and depends on the extent of the cancer when medical treatment begins. Results of this continuing study were reported by Dr. John Ziegler, NCI scientist and director of the Uganda Cancer Institute, at the NCI-sponsored Vth International Symposium on Comparative Leukemia Research in Padua, Italy, in September.

Since 1967, 130 patients with Burkitt's lymphoma have been treated with cyclophosphamide at the Center. Over 90 percent of this group achieved complete disappearance, temporarily at least, of their cancer. Of those with localized disease (usually facial tumors) 76 percent survived at least two years and of those with abdominal tumors 63 percent survived at least two years. Patients with involvement of the central nervous system on admission had a survival rate of 52 percent at one year. The poor survival rate of this group reflects in part the present inability to cure malignant invasion of the brain.

New Leads in Cancer Chemotherapy

In the February 1971 issue of the Proceedings of the National Academy of Sciences, Drs. George Johnson, Ira Pastan, and co-workers, had reported that adenosine 3',5'-cyclic monophosphate (cyclic AMP) and its derivatives partially restore normal cell characteristics to certain animal and human cancer cells grown in tissue culture. Cyclic AMP is found in all cells and mediates the action of many hormones.

Drs. Johnson and Pastan reported in the December 1971 issue of the Journal of the National Cancer Institute that prostaglandins, hormone-like compounds which also act with the help of cyclic AMP, caused changes in the structure and growth of certain animal cancer cells similar to the changes produced in the earlier study with cyclic AMP derivatives. These findings suggest that at least some actions of prostaglandins on cancer cells are due to increased levels of cyclic AMP stimulated by the presence of the prostaglandins. Clarification of the relationship between cyclic AMP and prostaglandins may possibly lead to control of tumor growth in man by administration of either of these two compounds in order to raise the level of cyclic AMP in the cancer cells.

Treatment with Surgery and Radiation

In recent years cryosurgery, the destruction of tissue by freezing, has been used in the palliation of readily accessible human cancers of the mouth, pharynx, prostate, and rectum. As a curative treatment it has been limited to skin cancers. But recent studies have encouraged investigation into cryosurgery as a potentially useful form of treatment for other cancers. In one such study, cryosurgery was found to eradicate liver cancers in rats, without adverse effects to the animal as a whole. These findings were reported in the November 1971 issue of Cancer by NCI scientists Drs. H. Bryan Neel, III, Alfred S. Ketcham, and William G. Hammond.

Surgical Treatment of Cancer of the Cervix

A review of NCI's clinical experience during a 15-year period with cancer of the uterine cervix, one of the most common forms of cancer in American women, was completed in 1971. Various aspects of this experience were reported by Dr. Alfred S. Ketcham and his colleagues in the May 1971 issue of Obstetrics and Gynecology and the November 1971 issue of Cancer.

This experience has resulted in the establishment of guidelines for treatment of patients with unusually large or incurable untreated tumors and of patients who have had previous treatment failure. Now such patients often can be treated with radical surgery safely and sometimes can be cured.

From 1954 to 1969, 162 patients with far-advanced cancer were treated with pelvic exenteration, the surgical removal of the pelvic organs and lymph nodes, resulting in a cumulative five-year survival of 38 percent. During the same period, another 84 patients with less advanced stages of cancer, were treated with radical hysterectomy, the surgical removal of the uterus and all or part of the vagina plus the pelvic lymph nodes (a more limited operation than pelvic exenteration), with a cumulative five-year survival of 83.5 percent.

Classification of Hodgkin's Disease Stages

At a conference in April in Ann Arbor, Michigan, on Staging of Hodgkin's Disease, a new method of classifying the stages of the disease was proposed to assist physicians in choosing the most effective therapy. In the November 1971 issue of Cancer Research, this new classification, or staging, method was reported by Dr. Paul P. Carbone of NCI and other members of the Hodgkin's Disease Classification Committee appointed by the American Cancer Society.

The new staging method is based on two systems of classification--clinical and pathological--rather than on the single clinical system of the classification method used since 1965. The need for a pathological system became apparent during the past few years when laparotomy (surgical exploration of the abdoman) was proposed as part of the diagnostic evaluation of Hodgkin's disease patients. Laparotomy allows a physician to obtain tissue samples for microscopic study (biopsy) from organs and sites not accessible to palpation and not clearly defined by X-ray examination.

The new staging method promises to improve the outlook for Hodgkin's disease patients, particularly for those with localized cancer in areas outside the lymph system. More effective comparisons of data from different cancer centers will also now be possible.

Immunology and Cancer

In a further step toward understanding the way the body fights diseases such as cancer, Dr. D. R. Davies and his colleagues in the NIAMD and Dr. William Terry of the NCI presented new data on the three-dimensional structure of a human antibody. Antibodies are proteins formed as part of the immune response against foreign agents called antigens, composed, for example, of cancer-related proteins. According to present concepts, a person may develop cancer when his immune system is no longer effective in recognizing as foreign the antigens of cancer cells and therefore does not proceed to deactivate the harmful cells.

In two reports in the December 10 issue of The Journal of Biological Chemistry, the investigators and their colleagues described the use of X-ray diffraction and electron microscopy to study the structure of a crystal of a human antibody, a protein called gamma G1 immunoglobin. These preliminary studies offer direct evidence that the overall outline of the molecule has either a T or Y formation. Further experiments are planned to determine the three-dimensional structure in greater detail and thus provide more information on the way antibodies interact with antigens.

Specific Antigens on Leukemia Cells

During the year NCI scientists presented evidence suggesting that leukemi cells contain specific antigens that make those cells distinguishable from normal cells. Establishing the presence of these antigens is an important step in developing therapy based on principles of immunology as well as detection methods. Such antigens may indicate that an individual has early, presymptomatic leukemia or that he has been exposed to the causative agent, possibly a virus, and may later develop the disease. These antigens thus may also serve as a marker for the causative agent.

In December 1971, Dr. Dean Mann and his colleagues at the NCI reported in the journal Science evidence for the existence of a specific antigen on human leukemia cells from a different type of test. Using antiserum from rabbits immunized against Burkitt's lymphoma cells, they tested the peripheral white blood cells of 15 patients with either acute lymphocytic or acute myelocytic leukemia, 41 of their relatives, and 527 unrelated persons. White blood cells of 8 of 15 leukemia patients and 4 of their relatives showed a positive reaction to the antiserum, suggesting the presence of a common immunologic factor among the patients and some of their close relatives. Most of the patients whose cells did not react were in remission, a temporary disease-free state. No reaction was observed with random individuals.

Evidence for the existence of specific antigens on leukemia cells, as indicated in these studies, suggests that it may be possible to detect persons exposed to factors that cause leukemia in order to plan for preventive or therapeutic measures.

How Tumors Grow

NIH-supported scientists at the Harvard Medical School and Children's Medical Center, Boston, interested in the immunological aspects of cancer, have found that a tumor itself contains an apparently distinctive agent which stimulates development of the capillaries necessary for continuation of its own growth. This substance, which the investigators have named TAF, or tumor angiogenesis (development of blood vessels) factor, has been isolated from both human and animal solid tumors. Thus far, it has not been found in non-malignant tissues.

The Boston scientists (Drs. J. Folkman, E. Merler, C. Abernathy, and G. Williams) suggest that, if TAF were to be blocked, tumor growth might well be arrested at a very early stage. Since, unlike normal tissue, only a growing tumor demands new capillaries for its continued growth, an anti-TAF agent (assuming one can be developed) would not be harmful to normal tissues but would have the potential to deprive cancer cells of a vital means for their continued expansion.

Research Information

Public interest in the Institute continued at a high level during the year, generated by a number of developments, particularly those mentioned at the beginning of this report: appropriations and funding; The National Cancer Act of 1971; the appointment of Dr. Frank J. Rauscher, Jr. as Director, NCI; appointments to the President's Cancer Panel and the National Cancer Advisory Board; and the acquisition by NCI of Fort Detrick facilities.

Inquiries on these and other subjects--from the Congress, the news media, and directly from the general public--continued to tax the facilities of the Research Information Branch which nevertheless maintained its function of originating news and information from the Institute by means of news releases, contacts with radio and television outlets, reports, staffing of news rooms at major scientific meetings, and other methods of increasing NCI visibility.

During calendar year 1971, 600 inquiries by newsmen were handled, 21 news releases and 29 press summaries were prepared, and 7 press rooms were staffed. A total of 14,400 general public inquiries were answered. Responses to White House and Congressional inquiries totalled 200 telephoned and 300 written. Research Information Branch publications numbered 33 titles in stock, of which 100,000 copies were distributed by the Branch, in addition to sales by the U.S. Government Printing Office. Four texts were updated and one new publication was prepared during the calendar year.

In the same period, four fact sheets and 30 reports, including materials for the Congress and the White House, were prepared. Nine bookings of the exhibit, "Research and Related Programs of the NCI," were made, and four displays were prepared. Institute staff members made appearances on five television and seven radio programs, and there were six interviews with NCI scientists for the "Men and Molecules" radio series of the American Chemical Society. The one-hour color television film, "Progress Against Cancer," was prepared for 1972 telecast. There were 18 showings of existing NCI films and filmstrips. Fifteen speeches and speech inserts were prepared, and 16 articles, including a 6,675-word article for the new Encyclopaedia Britannica were developed.

Reorganization Items

The Office of Associate Director for Extramural Activities was reorganized, with the following components:

Grants Administration Branch
Grants Management Branch
Grants Financial Section
Grants Processing Section
Administrative Services Section

The Laboratory of Pathology was relocated organizationally, and now reports to the Associate Scientific Director for Clinical Research, instead of to the Scientific Director, General Laboratories and Clinics.

The SALES Section of the Special Virus Cancer Program, responsible for the review and management of research on animal model systems, was terminated.

A Breast Oncology Section was established in the Surgery Branch.

A Research Contracts Branch was established in the OD-NCI, with the following components:

Chemotherapy Contracts Section
Etiology Contracts Section
General Laboratories and Clinics Contracts Section

The Office of Associate Scientific Director for Program Chemotherapy, was established.

A Drug Liaison and Distribution Section was established in the Cancer Therapy Evaluation Branch, Chemotherapy.

The Clinical Trials area, Chemotherapy, was renamed the Clinical Oncology area, and reorganized.

The Office of the Associate Scientific Director for Clinical Trials was renamed the Office of the Associate Scientific Director for Clinical Oncology.

The Medicine Branch as previously constituted was abolished, and its programs were reorganized as follows:

The Solid Tumor Service was made a Branch and named the Medicine Branch;
The Leukemia Service was made a Branch and named the Hematology and Supportive Care Branch;
The NCI-Veterans Administration Medical Oncology Service was made a Branch; its name remained the same;
The Human Tumor Cell Biology Branch was moved from the Clinical Oncology area to the Experimental Therapeutics area, Chemotherapy.

Staff Changes, Activities, Honors and Awards

Dr. Anthony M. Bruno was appointed Assistant Director, NCI.

Mr. John E. McShulskis was appointed Chief, Systems and Operations Planning Branch, Extramural Activities.

Dr. James A. Peters was appointed Deputy Scientific Director, Etiology, NCI; and in May 1972 was appointed Acting Scientific Director, Etiology.

Dr. Alfred S. Ketcham was appointed Clinical Director, NCI.

Mr. James F. Kieley retired as Chief, Research Information Branch, OD-NCI. Miss Jane Collins was named Acting Chief of the Branch in November 1971.

Mr. Charles E. Leasure, Jr., was appointed Administrative Officer for the Chemotherapy Program, NCI.

Dr. Vincent T. Oliverio was appointed Acting Associate Scientific Director for Experimental Therapeutics, Chemotherapy.

Mr. Leo F. Buscher was appointed Chief of the newly established Grants Administration Branch.

Mr. Thomas L. Kearns was appointed Administrative Officer for the Office of the Director, NCI.

Mr. Richard Sherbert was appointed Management Analyst, NCI.

Dr. William G. Hammond was appointed Chief, Clinical Investigations Branch, Extramural Activities, NCI.

Dr. Diane J. Fink joined the Institute as Program Director for Chemotherapy, Extramural Activities.

Dr. Gio B. Gori, Associate Scientific Director for Program, Etiology, was appointed Chairman of the Tobacco Working Group, succeeding Dr. Carl G. Baker.

Mr. George A. Brandner retired in January 1972 as Research Contracts Branch Chief, NCI.

Mr. Carl A. Fretts was appointed Chief of the newly established Research Contracts Branch, OD, NCI.

Dr. Seymour M. Perry was appointed to the newly established post of Associate Scientific Director for Program, Chemotherapy.

Dr. Paul P. Carbone was appointed Associate Scientific Director for Clinical Trials, Chemotherapy, succeeding Dr. Perry.

Dr. William W. Payne, deputy director of NIEHS, Triangle Park, North Carolina since 1967, was named senior project officer for NCI's cancer research center at Fort Detrick, Maryland.

Mr. Orley R. Bourland, Jr., a chemical engineer associated with Fort Detrick for 23 years, was appointed administrative officer for the new cancer facility there.

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Dr. George J. Todaro, Chief of the Viral Leukemia and Lymphoma Branch, Etiology, were presented the DHEW Superior Service Award.

Dr. Edward L. Kuff, Acting Chief, Laboratory of Biochemistry, General Laboratories and Clinics; Dr. Richard A. Malmgren, Head of the Cytopathology Section, Laboratory of Pathology; and Dr. Donald P. Tschudy, of the Metabolism Branch, General Laboratories and Clinics, were awarded the PHS Meritorious Service Medal.

Mr. Horace C. Turner, of the Viral Carcinogenesis Branch, Etiology, was presented the DHEW Forty-Year Length-of-Service Award.

Dr. Jacqueline Whang-Peng, of the Human Tumor Cell Biology Branch, Chemotherapy, received the Arthur S. Flemming Award for her contributions to the study of cytogenic defects in disease states.

Mr. Douglas G. Christian, of the Patient Care Pharmacy, Baltimore Cancer Research Center, Chemotherapy, was the recipient of the American Pharmaceutical Association's Military Section Literary Eighth Annual Award.

Dr. Stephen B. Guss and Messrs. William Idler and Paul Montague, of the Dermatology Branch, General Laboratories and Clinics in collaboration with Dr. Arthur R. Ugel of New York University were given the Gold Award by the American Academy of Dermatology for their exhibit, "Isolation and Characterization of Bovine Keratohyalin."

Dr. Gary L. Peck, Dermatology Branch, NCI, was awarded first prize in the 1971 North America Clinical Dermatological Memorial Essay Contest for his paper, "Toxic Epidermal Necrolysis in a Patient with Graft-versus-Host Reaction."

Dr. Abraham Goldin, Associate Chief for Laboratory Research, Drug Research and Development, Chemotherapy, received the Pierkinje Prize for contributions to chemotherapy, at the 7th International Congress of Chemotherapy in Prague, Czechoslovakia.

Dr. Kenneth M. Endicott, former Director, NCI, received a special award from the American Association for Cancer Research for his work as "an outstanding pathologist, scientist, and public health officer" in combating cancer through chemotherapy and in establishing NCI's viral cancer program.

Dr. Howard M. Temin, of the University of Wisconsin (an NCI grantee) received the 1972 Bertner Award for outstanding achievements in cancer research.

The Silver Certificate of Merit of the National Medical Association was received by NCI for an exhibit, "National Cancer Institute--Research and Related Programs," arranged by the Publications, Visuals, and Reference Section of the Research Information Branch, NCI.

The Van Meter Prize for achievement in the field of thyroid research was won by Dr. Ira H. Pastan, Chief, Laboratory of Molecular Biology, at the 1971 meeting of the American Thyroid Association.

Dr. Peter J. Deckers won two national first prizes while serving as a clinical associate in the Surgery Branch, NCI: one from the Student American Medical Association for his work on "The Adoptive Transfer of Concommitant Immunity to Murine Tumor Isografts with Spleen Cells from Tumor-Bearing Animals;" the second, the James Ewing Society Residents Award, for his research, "RNA-Mediated Transfer of Tumor Immunity--a New Model for the Immunotherapy of Cancer."

Dr. George J. Todaro, Chief of the Viral Leukemia and Lymphoma Branch, Etiology, received the Gustav Stern Award, during the eighth biennial Gustav Stern Symposium on Perspectives in Virology, for outstanding accomplishments in virus research.

Program Activities Report
Fiscal Year 1971-72
Editorial Office of the National Cancer Institute

The Board of Editors reviewed 1,300 items for publication in various journals. Those not destined for the Journal of the National Cancer Institute constituted 634 manuscripts and 267 abstracts and book reviews.

Of 399 manuscripts submitted for publication in the Journal of the National Cancer Institute, 66 were by NCI authors: 45 accepted; 3 rejected; and 18 pending. Authors from other research institutions submitted 333: 176 accepted; 53 rejected; and 104 pending. Of these, 109 were received from other countries.

Volumes 46 and 47 of the JNCI totaled 2,774 double-column pages.

One NCI Monograph totaling 298 pages was published: #34 Prediction of Response in Cancer Therapy.

To accommodate requests for rapid publication, a new section "Brief Communications" was opened. Short, timely articles of a nature and substance that permit other investigators to utilize the information, can be published within 16 weeks. Editorials, Highlights of meetings and Letters to the Editor were solicited.

ANNUAL REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM PLANNING AND ANALYSIS (OADPPA)
NATIONAL CANCER INSTITUTE
July 1, 1971 - June 30, 1972

Office of the Associate Director

The planning and analysis activities performed by the staff of OADPPA fall into two categories: the formulation of program and operational plans for new research efforts; and the revision and updating of ongoing programs based on research results. The approach to planning as a dynamic process, and not single discrete activities spaced in time, provides the planning staff with a continual base for relating and working with the scientists and managers in the operating programs. All planning activities are conducted by planning teams consisting of members of OADPPA and scientists (Federal and non-Federal) working in the area to be planned.

During FY 1971, an unprecedented planning effort was initiated to respond to President Nixon's call for an intensified and expanded national cancer program. A series of 40 planning sessions were held at Airlie House, Warrenton, Virginia, between October 1971 and January 1972. These meetings were attended by 250 clinical and non-clinical scientists for the purpose of developing scientific recommendations for the accomplishment of seven key cancer program objectives for the significant reduction in the incidence, morbidity and mortality of human cancers.

The OADPPA staff had the primary responsibility for the organization and conduct of these planning sessions with major contributions and help from the NCI senior staff.

The initial analysis of the over 3000 pages of material developed during the planning sessions will result in an overall program strategic plan for accomplishing the program objectives, and a program operating plan for implementing the strategic plan.

More detailed discussions of the planning effort are included in the Branch reports.

Mrs. Jacqueline Parkman, a Senior Scientist at Booz Allen Applied Research, joined the OADPPA staff as a member of the Systems and Operations Planning Branch.

Mr. Richard Holt, Vice President, Operations, Wolf Research and Development Corporation, joined the OADPPA staff. In addition to participating in the general planning and analysis activities, he brings special competence and experience in the development of management systems for large-scale programs.

ACTIVITIES OF THE PROGRAM ANALYSIS AND FORMULATION BRANCH (PAFB)

This Branch is concerned primarily with substantive matters in cancer research and in related matters. It prepares position papers outlining alternative approaches, guidelines and procedures and devises models for the review, evaluation and implementation of NCI programs. The Branch participates in discussions and prepares reports and documents on progress towards major objectives and identified needs of the Institute. These discussions include in-depth analysis of current programs, initiation of major program modifications and the development of outlines for new programs. An integral part of the Branch's operations concerns identification, review and analysis of resource requirements for NCI programs and the development of the plans, approaches and guidelines contributing to their implementation.

The members of the Branch provide assistance to the Director, the Scientific Directorate, the National Cancer Advisory Board and the Chiefs of the different line areas of the Institute on matters relating to research planning as described, e.g., development of structures and guidelines for formulating new research programs or for amending existing ones; development of logic arrays for new programs. The Branch also maintains liaison with individuals and groups outside the NCI on matters relevant to cancer.

Specific activities engaged in by the Branch during the past year are provided in the paragraphs that follow:

I. Development of a National Cancer Program Plan

The primary responsibility of the Program Analysis and Formulation Branch for the above time period concerned preparations for and participation in the development of a National Cancer Plan in response to the President's request for an intensified effort in cancer. The Branch (Drs. Cantarow and Klein) participated in the following activities incident to the development of this Plan:

A. Selection of an outside contractor to assume responsibility for supporting services.

B. Preparation of prospective lists of participants for the forty-one sessions to assure adequate representation for different disciplines and areas of interest.

C. Recruitment of participants.

D. Development of guidelines and background materials for the contractor in connection with the conduct of meetings of scientists and other professionals.

E. Development of general instructions and planning guidance materials for use by the chairmen and other participants at the sessions.

F. Analysis of objectives prior to presentation at the sessions, and development of suggested topics for discussion under each.

G. Provision of assistance to session participants and coordinators during the course of the scientific meetings.

H. Review and editing of reports during and subsequent to sessions.

I. Analysis of project area summaries prepared during the sessions. Review of abstracts and modification of approach elements and approaches based on these summaries.

J. Review of draft reports of the scientific sessions culminating in preparation of a final Executive Summary Report.

II. Development of National Organ Type Tumor Programs

A. National Program on Cancer of the Large Bowel

The Program Analysis and Formulation Branch (Dr. Klein) provided planning leadership in the development of a Logic Diagram portraying overall plans for a National Program in cancer of the large bowel. These completed plans are to be presented to the National Cancer Advisory Board at the June 1972 meeting.

B. National Program on Cancer of the Urinary Bladder

The Program Analysis and Formulation Branch (Dr. Klein) assisted Dr. Ellwein in the Systems and Operations Planning Branch, OADP, in developing a research logic plan for cancer of the urinary bladder comparable to the one on the large bowel. The completed plan was presented at the March meeting of the National Cancer Advisory Board and approved for funding under the Grants Program of the NCI.

III. Status of Research in Immunology-Immunotherapy and Recommendations for Expansion

In continuation of efforts initiated at the NCI the previous year, the Program Analysis and Formulation Branch (Dr. Klein) contributed to the efforts of the Immunology-Immunotherapy Study Group in developing a final report on the status of research in this field of cancer. This report was presented to the NCI Scientific Directorate July 1971 and led to the initiation of plans for development of a national program in immunology.

IV. Suggestions for National Cancer Program Contributed by Individual Scientists

Dr. Cantarow answered over a hundred letters received in response to Dr. Baker's letter in SCIENCE soliciting suggestions from the scientific community for types of research that should be included in the National Cancer Program.

V. Workshop on Carcinogenesis

Dr. Cantarow served as the NCI representative to the Core Group of the Workshop on Carcinogenesis being conducted under a grant to the Interdisciplinary Communications Program of the Smithsonian Institution. Two workshops were held during the past fiscal year, October 17-20, 1971 and May 24-27, 1972.

VI. Other Activities

Dr. Cantarow served as:

Member of the Board of Trustees, Jefferson Medical College
Member of the Policy Committee of the American Association for Cancer Research
Member of the Program Committee of the Seventh National Cancer Congress

Publications

Hoffman, S.A. and Cantarow, A.: Fatigue-Substance Inhibition of Transplanted Tumors, Fed. Proc. 30:652, 1971

ACTIVITIES OF THE SYSTEMS AND OPERATIONS PLANNING BRANCH (SOPB)

This branch is concerned with the development and application of systems and operations planning techniques to cancer research. It participates as members of planning teams organized to develop program plans for selected areas of the cancer problem; works directly with program and administrative personnel in the development of operations plans; participates directly with program personnel in the implementation phases of new programs; maintains effective liaison with program personnel; provides periodic consultation and direct effort, as requested by the Program leaders, to monitor, revise and update both program and operations plans; provides education and training to program staff in the use of systems techniques.

In addition to continuing its close working relationships with various program areas of the Institute by providing general management consultation and by participating in team planning efforts, the Branch staff was the focal point for the development of the National Cancer Program Plan.

The Branch Chief, Mr. John McShulskis, was the Project Officer for the effort to organize and conduct the planning sessions, analyze the session outputs and prepare the planning session documentation. He was a principal contributor to the preparation of the Planning Session Guidance Material, the Executive Summary and the Program Strategic Plan.

Dr. Leon Ellwein was assigned full responsibility for providing systems planning guidance for five major planning efforts to establish material programs in Breast Cancer, Detection and Diagnosis, Prostate Cancer and Bladder Cancer. In addition he provided assistance in the revisions of the Special Virus Cancer Program and Chemotherapy Program.

Specific activities engaged in by the Branch during the past year are provided in the paragraphs that follow.

I. National Cancer Program Planning Effort

As the primary focal point for the National Cancer Program Planning Effort, the Branch directed and participated in the following activities:

A. Prepared and updated the plan and schedule for the overall development of the National Cancer Program Plan (NCPP).

B. Participated in the preparation of the research hierarchy.

C. Prepared the Commerce Business Daily resource capabilities request for contractor support in providing administrative support services for the NCPP, and participated in the evaluation of the responses.

D. Prepared the Request for Proposal for the above services and assisted in the evaluation of the responses to select the contractor.

E. Arranged for the facilities (Airlie House, Virginia) for the NCPP planning sessions.

F. Organized,structured and scheduled the planning sessions.

G. Prepared the detailed Planning Session Background and Guidance Material for use by the 41 planning session panels.

H. Conduct pre-planning session briefings for the 41 planning session chairmen.

I. Provided guidance and consultation, when requested, at the planning sessions.

J. Compiled and analyzed the planning sessions output data.

K. Prepared comprehensive briefings on the NCPP effort for the Secretary, DHEW; Office of Science and Technology; Office of Management and Budget; Director, NIH; American Association of Cancer Institutes; President's Cancer Panel and National Advisory Cancer Board.

L. Prepared the format for the Priority/Resource Allocation Estimates and analyzed and formatted the results.

M. Prepared the five year Resource Plan for the NCPP.

N. Coordinated and participated in the preparation of the NCPP Executive Summary, and Program Strategic Plan.

O. Directed the administrative support services contractor in the

- organization and implementation of the planning session logistics arrangements (i.e., correspondence, travel arrangements, facility arrangements, etc.)

- coordination of the planning sessions
- compilation, preparation and publication of the 40 planning session reports
- preparation of all supporting documentation

P. Monitored and approved all contractual actions of the administrative support contractor and facilities contractor.

Throughout this effort considerable support in all of the above areas was provided by Mr. Hugh E. Mahanes, OD, Research Contracts Branch who is the Assistant Project Officer for the NCPP effort.

II. Development of National Programs in Organ-Type Neoplasms

The organ-type programs (Breast, Bladder and Prostate Cancer) are of national scope and encompass the prevention, detection and diagnosis, and treatment of each of the diseases.

The planning for the Breast Cancer Program was completed late in 1971 and the program is now being implemented under the management of the NCI Breast Cancer Steering Committee (Dr. Berlin, Chairman) of which Dr. Ellwein is an ex-officio member.

An intensive planning effort for the Bladder Cancer Program was initiated in September 1971 and after more than 30 planning meetings with inputs from over 40 biomedical scientists the plan was completed early in 1972 and received final approval for funding by the National Cancer Advisory Board at its March meeting. The scientific administration, including the coordination of the efforts of multiple grantees, is being carried out under the direction of Dr. G. Friedell at St. Vincents Hospital in Worcester, Mass. Dr. Price, Project Administrator (Extramural Activities) serves as the NCI liaison.

Concentrated planning for establishing a National research program on Prostatic Cancer began in April 1972 and is now proceeding under the scientific direction of Dr. G. Murphy at Roswell Park Memorial Institute.

The SOPB also worked with Dr. Klein (PAFB) in planning the National Program on Cancer of the Colon-Rectum.

III. Support in the Planning of Other National Programs

A major analysis and revision of plans for the Special Virus Cancer Program was completed in August 1971. The revised plan reflects the integration of research related to modification of the host with earlier plans for research based primarily on modification of etiological factors.

A group of NCI scientists under the direction of Dr. Terry (GL&C) have been charged with developing a national program in immunology. Mr. Carrese and Dr. Ellwein have worked with this group in formulating a plan which

reflects current key research objectives and explicitly identifies program decisions and decision criteria.

Dr. Ellwein has been working closely with Dr. Berlin (Scientific Director of GL&C) to develop a framework for a National program in Detection and Diagnosis of Cancer. Key problems along with approaches to solving these problems are being identified and include areas relating to general or non-organ specific detection and diagnosis as well as those peculiar to or relating primarily to a specific organ type. The planning of this program will continue by soliciting and integrating the inputs from members of the biomedical community actively working in this area.

As well as consulting with individuals on their specific problems, the SOPB has been engaged in a number of small planning efforts. Included in these activities is the development of a plan for a topical chemotherapy program.

IV. Institute Management Consulting and Department Planning Support

The branch is extensively involved in providing both management consulting support to all of the Program Areas in the Institute and planning support and coordination, the NCI aspects of department planning.

In the area of Institute management consulting the branch has been involved in the following activities:

- analysis of facilities construction and renovation/alteration alternatives (Chemotherapy)
- evaluation and selection of a biomedical engineering support contractor (Chemotherapy)
- evaluation and selection of an administrative support contractor (Chemotherapy)
- evaluation of a contract renewal for a Program Management System (Etiology)
- assistance in the preparation of a cancer center evaluation RFP (Extramural)
- evaluation and selection of a contractor for the Cancer Center study (Extramural)
- briefings to the staff on the Institute's planning efforts
- seminar on systems techniques

In the area of Department planning support the branch has been active in coordinating the NCI effort in the Operational Planning System and responses to the Department Planning Guidance Memorandum.

Publications and Presentations

Ellwein, L. B. and Gray, P., "Solving Fixed Charge Location-Allocation Problems with Capacity and Configuration Constraints", AIIE Transactions, Vol. III, No. 4, December 1971.

Ellwein, L. B., "Planning a Comprehensive Breast Cancer Research Program", presented at the 41st National Meeting of the Operations Research Society of America, New Orleans, April 26-28, 1972.

McShulskis, J. E., "Program Planning at the National Cancer Institute", presented to Industrial Research Institute, March 22, 1972.

SCIENTIFIC AND TECHNICAL INFORMATION OFFICE (STIO)

The major types of work performed by the Scientific and Technical Information Office (STIO) of NCI during Fiscal Year 1972 are listed below:

I. International Cancer Research Data Bank Activities

The STIO is deeply involved in planning and developments leading to the establishment of an efficient and effective International Cancer Research Data Bank (ICRDB). This activity includes major participation in meetings of an in-house steering committee which is guiding the developments of the ICRDB and the preparation of related reports and position papers.

The highlight of this activity was the establishment of a committee of outstanding national and foreign information scientists, library scientists, information systems managers, and information users to aid NCI in development of the ICRDB. The development of an agenda and background material for the first meeting of this committee in May, 1972, and the planning activities following the meeting, occupied large blocks of time in the first half of 1972.

II. National Cancer Plan Activities

The STIO participated in the processing of data related to the National Cancer Plan (NCP). This included development of computer programs that produced computer printouts containing the names of candidates nominated for participation in the NCP and related tabulated data (such as frequency of nomination by multiple sources of names).

In addition, several man-months were devoted to preparing abstracts of the 726 project areas proposed by participants in the NCP. These abstracts were processed by a specially-modified computer system to prepare one of the three major volumes describing results of the planning sessions.

STIO staff also participated in the initial planning sessions which preceded the NCP meetings.

III. Development of Methods for Indexing and Disseminating Cancer-Related Information

In anticipation of the interest in indexing and dissemination of cancer information, STIO has been active in the development of linear, enumerative hierarchical decimal classifications that will be useful for deep indexing of cancer-related data and for precise matching of cancer researchers and clinicians with this data. This development began almost a decade ago and has resulted in the development of an automated system which has been given the acronym "HICLASS" since it is based on HIerarchical CLASSifications.

Several years ago, the HICLASS system was first applied to the selective dissemination of information (SDI) to 103 cancer researchers in a one-year trial of a new type of computer matching system. This type of current-awareness service could supply scientists with a steady stream of useful abstracts describing publications related to their research.

During FY 1972, the results of that trial were summarized in a lengthy article that was published in Science. The article generated considerable interest and resulted in a significant level of correspondence with individuals interested both in the development and use of classifications in information systems and in the value of using these classifications for SDI systems. Additional technical comment on the HICLASS system was prepared and published in Science, in April, 1972.

A complex computer system called AUTOKLS (consisting of nearly 10 separate computer programs, some with almost 1,000 instructions) is being developed by STIO staff for easy production and updating of printed versions of the biomedical classification from data stored on magnetic tapes or disks. The programs use a small classification in the field of information science and technology as a sample data base to test and develop the system.

Before this system was developed, extensive retyping of individual pages or portions of pages were required to keep the classification abreast of developments in biomedical areas, and the problem of making changes in an alphabetical index to the classification and of keeping track of cross-references between categories was impossible.

The new system has solved many of these problems by linking categories in the classification with alphabetical terms referring to the categories. Using this new system, a change in the classification triggers a change or a change-message for the alphabetical index (and also triggers a change in any cross links to or from the changed category).

During FY 1972, three books containing hierarchical decimal classifications of information directly related to cancer and cancer research (in the fields of Therapy, Etiology, and Cancer Biology respectively) were prepared by using components of the AUTOKLS system. These preliminary

publications are based on computer printouts of the categories from magnetic tapes. The classifications are in various stages of revision and need much work, particularly to eliminate redundancy of some categories in classes 51.22, 51.32, and 51.46.

Steps have been taken to employ a skilled biomedical information specialist who will devote full time to improving and extending these classifications so that they will become even more useful for indexing cancer-related information and for matching cancer researchers with this data.

IV. Analyses of Cancer Research Supported by NCI

STIO has continued to design, develop, and operate computerized systems for analysis, indexing, storage, retrieval, and publication of information about NCI-supported research projects. The output of these systems is used by NCI administrators engaged in program analysis and program planning activities.

A Multi-Level Analysis of NCI Contracts by Scientific Area was published during FY 1972. Similar reports covering FY 1972 activities are in various stages of preparation.

Guidelines for preparation of abstracts used in these Analyses were developed and sent to organizations and individuals qualified to prepare abstracts. Methods were developed for rating the abstracting capabilities of these potential contractors, and four contractors have been selected from a group of eight to prepare abstracts in the coming year. When the contractor personnel are oriented and trained, they should be considerable help in preparing the abstracts of NCI-supported research projects that are required for in-depth analysis of NCI research programs.

Steps have also been taken to employ an additional staff member to assume responsibility for the preparation of these abstracts, the Multi-Level Analyses, and other technical reports originating in the STIO.

V. Computer Processing of Data for the Budget and Personnel Offices in OD/NCI.

STIO has developed a series of computer programs and systems based on the use of on-line computer terminals. These systems make it possible for secretaries and clerks in various OD/NCI offices to interact directly with data stored on magnetic disks at DCRT and to enter new or correct old data by very simple keyboard techniques. The data is used to generate both simple printouts and more sophisticated analyses of budget and personnel data.

The guiding principal in this system is to avoid the development of huge computer systems requiring constant care and maintenance and to develop individual programs and systems in a careful, incremental fashion as needs for new types of data arise. Only data that is clearly shown to be necessary for efficient operation of budget and personnel offices is entered into these systems, and extraneous information that has no immediate and definite use is excluded. Because of this studied simplicity, the development and alteration of programs to meet needs for new types of data and reports as they arise has been handled by a single, highly-competent programmer with minimum need for guidance or supervision.

Reports and listings produced for Budget and Personnel Offices are based on the following major types of programs developed by STIO:

A. Programs for preparing monthly reports listing all NCI personnel by organizational unit and giving data on grade and step level, salary, title and code number of position, etc.

B. Programs listing personnel by grade level or tabulating the overall grade level in NCI. These have proved very useful in connection with grade de-escalation requirements of NIH and in various types of manpower control activities in both the Budget and Personnel office.

C. Programs for maintaining information on training of NCI personnel and generating quarterly and annual reports of training that can be arranged in various ways (by organizational unit, by grade level of employees, etc.).

D. Programs for generating base budget and supplemental budget reports and for altering these reports to reflect shifting of funds between line items.

E. Programs for producing the publication "Who's Who at NCI" and for maintaining the basic data in a form that permits very easy updating of the text from year to year.

VI. Maintenance of a Computer System for Indexing of Experimental Cancer Pathology Data

In connection with Dr. Harold Stewart, a computer system has been designed and implemented for producing an index to slides and other data in the Registry of Experimental Cancers. In addition to designing the system the STIO provides continuing assistance in the input, correction, and processing of data. The indexes produced by this system give easy access to experimental animal tumor data that would otherwise be virtually inaccessible.

VII. Miscellaneous Activities

A. STIO has continued to provide information about scientific and technical activities of NCI to individuals and organizations (such as the American Cancer Society, the National Science Foundation, and the Federal Council of Science and Technology) on request.

B. STIO also serves as a focal point for collecting data, answering questions, and handling special problems related to information processing and publishing activities of NCI. This activity includes arranging for periodic meetings of key personnel engaged in information processing and data handling from all program areas at NCI so that mutual problems can be discussed and individuals can learn about information activities in other parts of NCI.

VIII. Publications and Other Professional Activities

A. Schneider, John H., "Indexing and Selective Dissemination of Scientific Information", Science 173, 300-308, July 1971.

B. Schneider, John H., "Selective Dissemination", Science 176, 434-437, April 1972.

C. Schneider, John H., Cancer Therapy (Preclinical and Clinical: A Decimal Classification (Categories 51.1, 51.2 and 51.3), National Institutes of Health, Bethesda, Maryland. April 1972. 57 pp.

D. Schneider, John H., Cancer Etiology and Selected Aspects of Cancer Pathology: A Decimal Classification (Categories 51.4 and 51.5), National Institutes of Health, Bethesda, Maryland. April 1972. 65 pp.

E. Schneider, John H., Cancer Biochemistry and Host-Tumor Interactions: A Decimal Classification (Categories 51.6, 51.7 and 51.8), National Institutes of Health, Bethesda, Maryland. April 1972. 32 pp

F. Schneider, John H., Multi-Level Analysis of NCI Contracts by Scientific Area, Fiscal Year 1971, published by the National Cancer Institute, October 1971. 75 pp.

G. Schneider, John H., Summary of Project Areas Proposed for the National Cancer Plan, published by the National Cancer Institute, February, 1972. 134 pp.

H. Schneider, John H., "The Case Against Input Cost Reduction and the Value of Hierarchical Classifications in Automated Information Systems." Presented ath the First Mid-Year Regional Conference of the American Society of Information Science in Dayton, Ohio, May 19, 1972.

I. Schneider, John H., "Hierarchies are Heavenly", presented as a member of a panel on "Current Concepts and Findings in Indexing" at a Workshop for Continuing Education in Library and Information Science in Washington, D.C., March 25, 1972.

J. Dr. Schneider was invited by the Brazilian Government to develop a set of plans and recommendations for improving the flow of scientific and technical information to Brazil from foreign countries. He met with various officials in the Division of Intellectual Cooperation in the Foreign Ministry in Brazilia and visited the major scientific information processing operations in Rio de Janeiro from November 12 to November 22, 1971.

K. Dr. Schneider has also served as Chairman-elect, and is currently Chairman of the Special Interest Group for Selective Dissemination (which is associated with the American Society for Information Science) during Fiscal Year 1972.

L. Dr. Schneider is also currently Chairman-elect of the Potomac Valley Chapter of the American Society for Information Science.

Contract Narrative
Office of the Associate Director for Program
Planning and Analysis, NCI
FY 1972

Contractor: Wolf Research and Development Corporation (NIH 71-22-70)

Title: Information Systems Consultation and Support for Biomedical Research - Task Order #5

Contractor's Project Director: Mr. Richard Holt

Project Officer: Mr. Louis M. Carrese

Objectives: Provide the necessary management systems analysis support to the Office of the Director, NCI, to assist in the establishment of requirements for the development and implementation of data management systems for the National Cancer Program.

Major Accomplishments: Reviewed existing management reporting systems within the Etiology, Chemical Carcinogenesis and Chemotherapy areas of the National Cancer Institute and analyze necessary interfaces between these systems and an overall NCI level management information system(s) and

Reviewed management reporting systems employed at various research installations both inside and outside the Government for applicability to the Conquest of Cancer Program, plus

Participated in arrangements and the development of discussion material for a meeting concerned with the formulation of initial plans for the establishment of an International Cancer Research Data Bank.

Significance to the National Cancer Program: The current and planned expansion of cancer research will require the continual analysis of data management systems to assure that these systems are responsive to both the scientific and operational needs of the program. This task provided an overview to current NCI systems and preliminary analysis of future requirements.

Proposed Course: There are no present plans to continue this task beyond the terminating date.

Date Contract Initiated: 5/3/71

Current Contract Level: 5/3/71 to 6/30/72
$31,366

Research Information Branch

Office of the Director

National Cancer Institute

Program Activities Report, July 1, 1971-June 30, 1972

On December 23, 1971 the President signed The National Cancer Act of 1971, climaxing a year of intense legislative efforts to structure a new national cancer program. The year's events had continuing impact on the Research Information Branch, as the public and news media sought information on the legislation and its interpretations, the National Cancer Advisory Board and its membership, the President's Cancer Panel, and on many diverse topics relating to cancer.

Close to 17,000 individual inquiries from the general public were answered by the Branch; more than 500 requests from the news media were handled; and dozens of news releases, reports, articles, speeches and publications were prepared--all by a Branch staff that averaged 20 persons during the year.

A National Cancer Program Plan (NCPP) and International Cancer Research Data Bank (ICRDB) were authorized by the new law, and Branch members prepared informational materials on their development, as well as assisting in preparation and planning efforts. (See individual Section reports for details.)

With the appointment on May 5, 1972 of Dr. Frank J. Rauscher, Jr. as Director of the National Cancer Institute, the news media expressed serious interest in the new Director's philosophy and program plans. A series of interviews with the Director were arranged for newsmen.

Like the rest of the Institute, the Research Information Branch was experiencing major changes. James F. Kieley, Chief of the Branch for 20 years, left NCI on November 1, 1971 to begin his retirement. Jane E. Collins was asked to serve as Acting Chief of the Branch starting November 1, a post she held during the greater part of the report year. The heavy demands placed on the Branch staff during this period of ferment in cancer research called upon all the resources and strengths of individual staff members, and seemed to result in a heightened esprit de corps. Branch members were consistently well-informed and responsive to program needs, thanks in part to a series of briefings arranged by the Chief and Acting Chief of the Branch. In the Fall of 1971, the NCI Director and the Associate Director for Program Planning and Analysis addressed the staff on separate occasions. These were followed by briefings on December 17, 1971 by the Executive Officer, NCI; on January 13, 1972 by the Financial Management Officer, NCI; on January 21 by the Chief, Systems and Operations Planning Branch, NCI; and on February 16 by the Associate Director for Extramural Activities, NCI.

Branch staff members kept well informed through familiarity with the scientific, medical and lay press, through attendance at major meetings and

conferences, and through close coverage of the NCI program areas. Staff members were individually assigned to cover events in the Chemotherapy, Viral Etiology, Chemical Carcinogenesis, Demography, General Laboratories and Clinics, and Extramural Activities areas.

Since the passage of The National Cancer Act of 1971, the Acting Chief of the Branch has established liaison with personnel in the Office of the Staff Assistant to the President for Health Affairs, and others in the White House, in addition to maintaining the usual lines of communication and responsibility with Information Offices in the National Institutes of Health and the Office of the Secretary, DHEW.

In December 1971 the Acting Chief presented proposals to the Director, NCI on public affairs activities urgently recommended to fulfill the new national cancer program plan. In addition, on December 30, 1971 she submitted to the Director, NCI recommendations for implementing those sections of The National Cancer Act of 1971 that relate to public affairs and information activities.

Branch staff prepared materials for hearings by the Subcommittee of the Committee on Appropriations, House of Representatives. This included the Opening Statement of the Director, NCI to the Subcommittee on the fiscal year 1973 appropriations; a statement by the NCI Director requesting a $40 million supplement for construction and training funds for use in fiscal year 1972; and a 17-page document, "Highlights of NCI Research during 1971."

The Institute's new 58-1/2 minute 16 mm. color film, "Progress Against Cancer" was approved by HEW and the White House in mid-November 1971, but was recalled by the White House on November 24 for further review. It was finally approved at a screening on February 18, 1972. At the request of members of the National Cancer Advisory Board, the Chairman of the President's Cancer Panel, Mr. Benno C. Schmidt, has made the film available to a major television network for possible telecasting in the coming months.

In addition to the 58-1/2 minute film, the RIB is also planning a 27-1/2 minute version for possible telecast and for distribution to schools, churches, and business and civic groups. Following a period of promotion and distribution by a contractor, continuing distribution will probably be arranged by the National Medical Audiovisual Center in Atlanta, Georgia. Requests to screen the film for telecasting have been received from the Gilmore Broadcasting Corporation and the Canadian Cancer Society.

Information and Education Section

In keeping with the mission of this Section--to adapt information on cancer research to the needs of the public--activities were carried forward on a number of fronts during the fiscal year, particularly in the areas of public, Congressional and news media inquiries, speech writing, radio and television relations, and regular coverage of the Extramural and Biometry-Epidemiology areas.

The enormous backlog of unanswered correspondence from the public, as described in last year's Annual Report, was brought under control, coincident with the addition to the Section staff of two professional writers who spend full time drafting replies to letters received here. However, the flow of incoming correspondence continues, and in appreciable numbers, demanding the constant efforts of the expanded staff.

A spate of public inquiries on any given subject are occasioned by any one of a number of causes: a news-media story on some aspect of cancer cause, prevention or treatment; an organized "write-in" campaign on the part of an external individual or organization; or public information emanating from the Institute itself, to cite just three examples. A case in point was the upsurge of letters to the President in the Spring of 1972, urging him to raise his fiscal year 1973 budget request to the Congress for the National Cancer Institute from $430 million to the $530 million authorized the National Cancer Institute for Fiscal Year 1973 by The National Cancer Act of 1971. This upsurge was the result of an organized campaign by the Citizens Committee for the Conquest of Cancer, and the White House forwarded such letters to NCI for reply. A spot check of these inquiries revealed that from March 1 to May 1, 1,507 letters in this category were received and answered. Other letter-writing campaigns were organized by the Citizens Committee for Cancer Survivors on Krebiozen; questioning the intended purpose of the President's Cancer Panel; and the International Health Council, inquiring about the Institute's authority to set safety standards for persons using anticancer materials, the composition of the National Cancer Advisory Board, and the testing of Laetrile. The two last-named campaigns produced inquiries to NCI at the rate of about one per day, and were continuing to be received as this report was written. In addition, one Senator (Robert Taft, Jr., of Ohio) disclosed that he had received a great many letters inspired by the International Health Council, and requested the Institute to furnish him a prototype reply. Inquiries in the many other categories pertinent to the cancer problem continued to be received as usual, with letters concerning treatment of cancer patients or cancer detection receiving high priority of attention.

With the expansion of the Federal cancer effort, the demands on the NCI Director to deliver speeches to interested groups around the country increased. An Assistant Director of the Institute complied with these speech requests on several occasions, and sought the aid of the Head of this Section in drafting the required talks. Several speeches were prepared in this manner during the reporting year.

The Section received and complied with eleven requests from the electronic media--radio and television stations and networks--to recruit scientific staff members for interviews on the air, panel shows, and the like. In an era when broadcast journalism continues to expand, this has resulted in considerably increased visibility for the Institute and its programs.

During the year a member of the Section was assigned to cover the activities of the Biometry-Epidemiology area, and to report on the program and its findings, as appropriate. In addition, the Section continued its coverage of the Extramural area--apprising the public, the news media, and the Congress

on developments in appropriations, funding, and such information on grants and contracts as is not privileged. This information was transmitted through correspondence, telephone inquiries, and visits by interested people.

Miscellaneous other activities were pursued by the Section during the year, including the preparation of news releases, NIH "Search for Health" newspaper columns, and reports, and the updating of cancer information pamphlets.

Assistance to News Media

The news media--newspapers, periodicals, radio and television--continued their inquiries during the year for information useful to the development of stories, and many of them were handled by the Section through the medium of telephone calls and visits by reporters. A total of 101 press calls was received. News releases were also prepared.

Requests for information came from such varied sources as the news-wire services--the Associated Press and United Press International--daily and weekly newspapers such as the Trenton Times, National Observer, Tampa Times, Medill Newspapers, Il Messaggero (Rome), Thomson Newspapers (England), Frederick Post, Washington Star, Scripps-Howard Newspapers, Washington Post, New York Times, Hagerstown Morning Herald, Daily Oklahoman, and the Dayton Journal-Herald. Special medical and science publications included Laboratory Management, Biomedical News, Drug Research Reports, Science News, Medical World News, Chemical and Engineering News, Nature, International Medical News, U.S. Medicine, Medical Research Engineering, Job Safety and Health Report, and Medical Tribune. Periodicals of general circulation serviced were Chilton Publications, Harper's Magazine, Newsweek, Time, Fortune, Family Circle, Good Housekeeping, and McGraw-Hill Publications. Book publishers with inquiries were Time-Life Books, and the World Book Encyclopedia. Electronic media included NBC Radio, CBS News (Walter Cronkite Show), the British Broadcasting Company, National Public Radio, Metromedia News (WTTG, Washington), and NHK (Japanese National Television). Other miscellaneous requests came from the Polish News Agency and the Washington Merry-Go-Round (Jack Anderson, syndicated column). A sampling of the subjects of these requests would show periodicals devoting entire issues or special sections to NIH or NCI; queries on appropriations and funding, cancer centers grants, marijuana carcinogenesis research, new or unproven cancer treatments, Institute-supported research findings at outside institutions, the history of cancer legislation, cancer incidence and mortality information, specific disease details, "clustering" of Hodgkin's disease patients, plans for the utilization of Fort Detrick as a cancer research facility, the National Cancer Plan meetings, the effects of diethylstilbestrol taken by pregnant women, the future of cancer control, compounds screened in the Chemotherapy area, research into a less hazardous cigarette, chemical carcinogenesis contracts, the cancer-causing effects of nitrosamines, progress of the testing of a platinum compound as an anticancer drug, and so on.

One interview with a freelance writer--a Fellow at the Washington Journalism Center--resulted in a feature article on the Institute in the

"Potomac" Sunday supplement section of the Washington Post. The article dealt with several aspects of the cancer effort, including legislation and funding.

The Section prepared a total of 56 news releases and articles during the year. These covered a variety of subjects: the appointments of Dr. Anthony M. Bruno as Assistant Director, NCI, and Dr. Alfred S. Ketcham, as Clinical Director; a minority conference sponsored by the Institute; and the awarding of a contract for a cancer center at the Mary Hitchcock Memorial Hospital in New Hampshire. There were articles on Hodgkin's disease and cancer survival rates prepared for the NIH Feature Service. NIH Record articles dealt with a variety of staff appointments, honors, awards; foreign visitors; research contracts; meetings, and so forth. A science writer from the London Daily Express spent four days at the Institute, conducting interviews with scientists, as arranged by RIB, with a view to writing a series of articles on cancer research, for British readers.

Public Inquiries

The Section answered 9,256 public letter and telephone inquiries. In addition to the subjects mentioned at the outset of this report, there were inquiries about proven or unproven methods of cancer treatment; unsolicited theories, methods, or materials for detecting or curing cancer; requests from students for cancer information or specimens for school science projects; requests from others for information on cancer, including bibliographies, or inquiries about the role of environmental or personal factors in the cause or prevention of malignant disease. A total of 6,512 pieces of published cancer literature was sent out in response to these inquiries.

Congressional inquiries, written or telephoned to this Section, totaled 388, on a variety of matters. With the expansion in the Federal cancer effort, there were a large number of letters dealing with appropriations, funding, and requests from the Congressional constituency for assistance in obtaining grant or contract funds for individual projects. Other letters and telephone calls requested assistance, both medical and financial, for cancer patients, information on unproven remedies and detection methods, and offers to serve on the National Cancer Advisory Board.

Miscellaneous Activities

In addition to the speeches mentioned above, on the cancer problem, a speech was prepared for Representative Samuel L. Devine, of Ohio. The Section Head was assisted by a GS-11 staff member in the preparation of some of the foregoing material. During April, when some members of Congress were called upon to address "kickoff" cancer drive meetings, several administrative assistants to legislators were given guidance by the Section in writing speeches for their superiors. Of the 16 "Search for Health" newspaper columns prepared in the Section, five were written for release in papers during April, annual Cancer Month by Presidential proclamation.

Three staff members joined the Section staff during the year--two writers, and a stay-in-school clerk-typist. One GS-11 writer left the staff at the termination of his temporary appointment. The Section Head completed the NIH Senior Seminar for Supervisors. Two staff members attended the NCI Equal Employment Opportunity Conference at the Annapolis-Hilton Hotel, Annapolis, Maryland. A member of the Section was assigned to write copy for NCI's EEO Newsletter.

The Section Head drafted two messages for the President's signature--one to the United Order True Sisters on the occasion of the organization's 125th anniversary, and the Columbus (Ohio) Cancer Clinic, on its fiftieth anniversary. A Spanish version of the Section's "Leukemia Fact Sheet" was prepared and reproduced for mailing where appropriate. Twenty-six pharmacology students and faculty members from the University of Gent, Belgium, were received and given a briefing and tour in the Institute's Drug Research and Development area, arranged by the Section. A Section member drafted an updated version of "Progress Against Leukemia," a pamphlet for distribution to the general public. A fact sheet on Federal funding of cancer research was prepared by a member of the staff.

Research and Program Reports Section

The Section's responsibility is to inform the public, the media, the medical community and the Congress of scientific developments in the field of cancer research. The Section is charged with obtaining and disseminating this information in the most meaningful way to its various audiences.

Accordingly, Section staff spent substantial time acquiring information on the NCI research programs which they have been assigned to cover: chemotherapy, immunology, virus research, chemical carcinogenesis; the NCI intramural research program designated General Laboratories and Clinics, and the National Cancer Program Plan.

Because of the extraordinary events of this past year bearing on the future organization, direction, and funding of greatly expanded cancer research activity, the thrust of the work of this Section has expanded to include these subjects.

Throughout the year, the Section prepared materials for its various audiences and answered their specific requests. The Section Head, 3 writers, one scientific reference analyst, and one secretary--

--responded to 400 press calls
--prepared 22 news releases
18 press summaries
12 weekly reports
5 Fact Sheets
Some Cancer Facts
Brief History of Recent Legislative Activities Concerning

an Expanded National Cancer Research Program
Drugs Used Against Cancer (updated as of April 1972)
Cancer Centers Program
Fort Detrick

--revised and updated 5 publications
Treating Cancer
Cancer - What to Know, What to Do About It
Cancer of the Mouth
Cancer of the Colon and Rectum
Cancer of the Skin

--wrote speech material for the Secretary of DHEW and the Surgeon General
--wrote a 1500-word magazine article for the Secretary
--staffed press rooms of 6 scientific conferences
First International Congress on Immunology
Vth International Symposium on Comparative Leukemia Research
Symposium on Hodgkin's Disease
Conference on Planning for Cancer Centers
American Association for Cancer Research
National Conference on Cancer Chemotherapy

--attended 4 conferences to assist the press or to cover for reports or NIH Record stories
Sixth Joint Working Conference of the Special Virus Cancer Program Meeting
Symposium on Mammary Carcinoma
Breast Cancer Therapy
Lung Cancer

--attended 4 conferences for background
Second Joint Working Conference of the Chemotherapy Program
Airlie House meeting for NCPP Planning
Cancer of the Pancreas Conference
Airlie House meeting for International Cancer Research Data Bank Planning

--scanned 350 journals each month

--noted and recorded 78 references each month

ASSISTANCE TO THE NEWS MEDIA

Because of the extraordinary events of this past year relating to the organization, direction, and funding of a greatly expanded cancer research activity, the scope of the work of this Section has markedly expanded.

Press Calls and Response

Members of the Section responded to 400 calls from the press during the year. During the peak month, December, The National Cancer Act of 1971 was

signed by the President; Airlie House meetings of outside scientists to develop the National Cancer Program Plan were taking place; a Conference on Planning for Cancer Centers was held; public interest in Fort Detrick as a National Cancer Institute research facility was accelerating; and research findings on candidate human viruses were announced early in December in California and Washington, D. C.

Throughout the year Section members assisted newsmen in gathering information for "round-up" stories for such periodicals as Fortune, Business Week, Life, Reader's Digest; newspapers including the Wall Street Journal, the National Observer, the Boston Globe, the Baltimore Evening News, the Philadelphia Bulletin; electronic media such as CBS News, NBC, VOA; and USIA. These media were focusing on cancer research findings, on the new cancer legislation, goals of expanded research activity, and planning of greatly augmented research programs.

During the Fall of 1971, while the legislation for agreatly expanded cancer research effort was being hammered out in Congress, the press and public sought details of the various legislative proposals. In answer to these requests, the Section prepared a legislative chronology--A Brief History of Recent Legislative Activities Concerning an Expanded National Research Program.

Draft of a news release summarizing The National Cancer Act of 1971 was prepared by the Section at the request of the White House for the signing ceremony. This material and excerpts from the legislative chronology were incorporated into a White House Fact Sheet on The National Cancer Act of 1971, distrubuted by the White House.

In responding to inquiries on the new legislation, Section members individually assisted journalists from the New York Times, the Detroit News, the Wall Street Journal, Science Magazine, the Medical Tribune, Science News, Voice of America, and others.

As implementation of the new legislation began, press inquiries regarding the President's Cancer Panel and the new National Cancer Advisory Board mounted. Distribution was made by the Research Information Branch to a mailing list of 2,370 of a January 31 White House release announcing the President's appointment of Dr. Robert A. Good and Dr. R. Lee Clark to the Panel. The appointment of Benno C. Schmidt as Chairman of the Panel had been announced in the President's Statement at the signing of the Act.

Beginning in April 1972 press calls were received by Section members regarding the appointment of a new director for the NCI. The New York Times, the Washington Post, Nature, Science, the Blue Sheet were among the first of the media making inquiries on this subject. Upon the appointment of Dr. Rauscher, in May 1972, requests for interviews continued and several press conferences were arranged.

Press Interest in the National Cancer Program Plan(NCPP)

As a corollary to interest in the new legislation, members of the press and public sought information on plans for the expanded national cancer research

effort. Begun last year with the articulation of goals and objectives for the National Cancer Program Plan, a massive planning effort involving 250 scientists was launched. Section members attended a portion of the October 1971 NCPP meeting for orientation in the method of Plan development and the November 19, 1971 session of the meeting chairmen. Six news releases on the NCPP planning sessions were prepared by the Section between October 1971 and March 1972. A Fact Sheet on the NCPP is in preparation.

Inquiries about the NCPP were received from the Associated Press, New York Times, Wall Street Journal, Los Angeles Times, Business Week, CBS News, New York Daily News, Science, Baltimore Evening Sun, Medical World News, Blue Sheet, Biomedical News, Chemical and Engineering News, Mexican National TV (Mexico City), and the Tobacco Institute.

Press Interest in the International Cancer Research Data Bank(ICRDB)

In the winter and spring of 1972 planning for the development of an International Cancer Research Data Bank, a mandate of The National Cancer Act, (Sec.407), was also of interest to the press. The Section Head participated in preliminary ICRDB planning meetings in February and March 1972. A news release was issued on a NCI-convened meeting May 24-26 at Airlie House, of international communications experts in research data dissemination, serving as consultants in the development of the ICRDB.

Press Interest in Planning for Cancer Centers

Because The National Cancer Act (Sec.408) authorizes the establishment of "15 new centers for clinical research, training, and demonstration of advanced diagnostic and treatment methods," the subject of cancer centers received special attention from the press this year. Section members staffed the press room of a Conference on Planning for Cancer Centers, co-sponsored by the NCI and the American Cancer Society, December 8-10, 1971, at the Washington-Hilton Hotel. A Fact Sheet on Cancer Centers was prepared by the Section Head and copies of the papers by NCI speakers were made available to the press. A highlight of media coverage of the meeting was an interview of the NCI Director on Channel 5-TV news, arranged by a Section member. Newsmen attending the Conference included representatives from Business Week, the Chicago Sun-Times, the Buffalo Evening News, the Columbus, Ohio Dispatch, Washington Evening Star, Miami News, the "Blue Sheet," Voice of America, and WTTG/TV Metromedia. Conference papers were requested by United Press International, Washington Report on Medicine and Health, Science, Biomedical News, Medical Tribune, Chemical and Engineering News, JAMA, U.S. Medicine, Hospital Practice.

In March 1972 a news release was prepared by the Section on the award of a grant to the University of Alabama Medical School to support cancer research and training activities at a Regional Cancer Center being developed in Birmingham.

Press Interest in Fort Detrick

Beginning in October, NCI's acquisition of a substantial portion of the Army's Biological Defense Research Center at Fort Detrick occasioned a large number of press inquiries, especially from newspapers and radio stations in the Washington, D. C. and Frederick and Hagerstown, Maryland area.

A ten-page Fact Sheet on Fort Detrick was prepared by a member of the Section in January for the Fort Detrick Bidders' Conference, and distributed widely on request--for example, to the Associated Press, Scripps-Howard, Washington Post, Washington Evening Star, Baltimore Evening Sun, U.S. Medicine, Biomedical News, Frederick News Post, Hagerstown Morning Herald, National TV (Mexico City), and the National Highway Safety Commission, Department of Transportation. The Fact Sheet described the facilities (with a map of the property), the method of operating the facility by a private contractor, the handling of contract negotiations, details of transfer of buildings by the Army, the type of research to be done by the NCI contractor, and a projected timetable.

Biomedical Conferences

Assistance to NCI scientists and to the press at biomedical conferences in this country and abroad was provided by the Section.

At the First International Congress on Immunology, which met August 3-6, 1971 in Washington, the Section helped to staff the press room.

At The Vth International Symposium on Comparative Leukemia Research in Padua-Venice, Italy September 13-17, 1971 the Section Head shared responsibility for press coverage with a representative of the Leukemia Society of America. Prior to the Symposium, a great deal of advance work was done by the Section Head to stimulate interest on the part of the American news media to cover the event. As a result, a New York Post journalist, Barbara Yuncker, who acted as a pool reporter, sent a daily story by cable to her paper and the Associated Press. In addition, Judy Randal of the Washington Evening Star, science reporter, filed daily dispatches which also went on the New York Times wire serving more than 300 newspapers. Medical World News, Medical Tribune, Infectious Diseases, and a variety of Italian news media covered the meeting.

Eight press summaries of papers to be presented by NCI scientists were prepared by members of the Section, one a summary of four papers on "Chemical and Viral Co-Leukemogenesis." Other summaries discussed polymerase in human leukemia cells; RNA tumor enzymes; immune suppression and the induction of cancer by Moloney Leukemia Virus and murine sarcoma virus; immunochemical studies of the group-specific antigens of mammalian C-type viruses; oncorna-type viral envelope antigens of feline leukemia and sarcoma viruses; particles in a rhesus monkey tumor; and Burkitt's lymphoma. Before the conference inquiries were received from such news media as the Associated Press, Voice of America, U.S. Medicine, Biomedical News, Infectious Diseases, International Medical News. A news release and program of the Symposium were distrubuted jointly by the NCI and the Leukemia Society.

In October 1971, a Symposium on Hodgkin's disease, co-sponsored by the American Cancer Society and NCI in St. Louis, was publicized by the Section, via news release and the operation of a press room throughout the meeting. Four NCI press summaries were prepared: (1) prognostic indicators of Hodgkin's disease; (2) NCI radiation therapy studies; (3) the effect of combination chemotherapy on survival of patients with advanced Hodgkin's disease; (4) delayed hypersensitivity and lymphocyte transformation. Science writers from JAMA and Medical World News covered the sessions as did a noted European science writer, Professor Enrico Anglesio of La Stampa.

Local media also reported the event. The St. Louis Post Dispatch and St. Louis Globe Democrat carried stories on the meeting. The St. Louis outlet of CBS-TV telecast an interview with NCI scientist Dr. George Canellos on drug treatment in Hodgkin's disease. KPLR-TV, an independent, carried an interview with Dr. Henry Kaplan of Stanford on radiotherapy. KMOX-radio, a major outlet in the midwest, broadcast a 10-minute interview with Dr. Stephen K. Carter of NCI, followed by a 35-minute question-and-answer period. An overflow crowd of more than 500 physicians attended the meeting.

For the 162nd Annual Meeting of the American Chemical Society in Washington September 13-17, 1971, a press summary of Dr. C. G. Zubrod's paper on cancer chemotherapy was prepared by the Section. Another was prepared on a paper by Dr. Peter T. Mora of NCI, entitled "Effect of Halogenated Carbohydrate Derivatives on the Aminosugar Metabolism of Virally Transformed Cells."

A press summary for the 57th Annual Meeting of the Federation of American Societies for Experimental Biology on April 9-14, 1972 in Atlantic City, was prepared by the Section, describing the treatment of leukemic mice with adriamycin and methotrexate.

At the 72nd Annual Meeting of the American Society for Microbiology in Philadelphia, April 23-28, 1972, a press summary was provided, reporting the suppression of the cytopathic effects of herpesvirus saimiri by cytosine arabinoside.

For the annual meeting of the American Society of Clinical Oncology in Boston on May 3, 1972 a press summary on evidence suggesting a possible link between viral hepatitis infection and the development of primary liver cancer was prepared by a Section member.

In May, 1972, for the 13th consecutive year, Section members handled the press relations for the 63rd Annual Meeting of the American Association for Cancer Research, in Boston. Before the meeting a news release was issued; letters of invitation to scientists for formal press conferences were prepared; requests for press summaries of papers were dispatched; three press summaries of papers by NCI scientists were written by Research Information Branch staff. The Section Head and another member staffed a press room throughout the meeting.

On May 18-19, 1972 a chemotherapy seminar titled "Current Concepts in the Treatment of Malignant Disease" was held at the Clinical Center for general practitioners. The Section prepared an announcement and arranged other promotional materials.

On June 1-3, 1972 in New York City the Section Head and a Section writer shared responsibility for press relations with staff of the American Cancer Society at a NCI-ACS co-sponsored National Conference on Cancer Chemotherapy. Press releases were prepared and the press room was staffed by the Section.

Additional Press Releases

During the year other news releases on newsworthy cancer research were prepared. For example, a preliminary study of cancer incidence in 1969 in selected areas of the country--part of the Third National Cancer Survey--was described in an October 1971 news release and engendered a number of press inquiries, especially from geographical areas surveyed in the study. They included Time, the New York Times, the National Observer, the Detroit News, the Atlanta Constitution, JAMA, Scripps-Howard publications, WPVI-TV in Philadelphia, the Minneapolis Tribune, the Minneapolis Star, Science News, and Hospital Practice. When copies of the preliminary report became available, they were sent on request to the press and to scientists who learned of its availability through a notice in the November 1971 issue of the FASEB Newsletter.

Evaluation of a detection test for early cancer of the colon--the carcino-embryonic antigen test developed by Dr. Philip Gold--was the subject of a news release in July 1971. In September 1971 NCI contracts awarded to three other research institutions for research on cancer of the colon and rectum were described in another release. Also in September, the public was informed by news release about a new NCI-supported project to improve survival of lung cancer patients by applying early detection methods, to be conducted at the Mayo Clinic.

Research studies at an Israeli medical school on immunotherapy--bolstering the body's natural defense against cancer--were described in a January 1972 news release. In March 1972 an announcement was made on findings by NCI scientists that three new RNA viruses which may cause cancer in primates differ immunologically from similar RNA viruses found in the mouse, rat, hamster and cat. In April 1972 NCI's new liaison offices in Japan and Belgium, opened by the Chemotherapy Program to foster greater international cooperation in the development and use of anticancer drugs, were described in a news release. Late in the month a press release announced the success of three teams of NCI scientists in reversing the normal flow of genetic activity among fundamental cell chemicals to permit reconstruction of a portion of the gene for red blood cell protein.

Throughout the year the Section responded to press inquiries about various aspects of cancer research or about specific research projects. In July a large number of press calls were received regarding a report made in Houston by Drs. Leon Dmochowski and Elizabeth S. Priori of type-C particles found in cultured cells of a 5-year old boy with Burkitt's lymphoma. The investigators believed that the virus, named ESP-1, was the first successfully cultured human cancer virus. However, subsequent immunological studies suggest that the ESP-1 virus shares many characteristics of the mouse leukemia virus and may be a contaminant.

Subjects of other inquiries included rifampicin compounds; the 4-drug treatment for advanced lymphosarcoma; cancer diagnostic tests; reverse transcriptase; clustering of Hodgkin's disease patients; platinum compounds; total body radiation; BCG; and an evaluation of calusterone as a potential anticancer drug.

PUBLICATIONS, FACT SHEETS AND REPORTS

Five publications were revised and updated by Section members during the year. Treating Cancer, Cancer: What to Know, What to Do About It, and NCI site pamphlets Cancer of the Mouth, Cancer of the Colon and Rectum, and Cancer of the Skin.

Three booklets describing the NCI Baltimore Cancer Research Center were initiated by a Section member--one on patient admissions, another providing patients with information for in-hospital use, and the third on research activity there.

For the November 1971 meeting of the Council for the Advancement of Science Writing in Brookhaven, New York, an eight-page Fact Sheet was prepared. It included data on cancer incidence, cancer drug research (with an updated chart of Drugs Used Against Cancer), cancer-causing chemicals and natural products, cancer-causing viruses, and the Legislative Chronology mentioned earlier. The chronology was completed as a cancer bill,was voted upon in the House of Representatives, went to Conference, was finally passed by the Senate and House, and signed into law by the President on December 23, 1971.

Fact Sheets on the Cancer Centers Program and on Fort Detrick have already been described.

Reports have been written by Section members throughout the year on a wide variety of research findings. Subjects ranged from "Nucleotide and its Analogs Partially Reverse the Malignant Process" to a discussion of the development of rapid, sensitive assays of environmental carcinogens (both issued in July 1971), to "Virus Isolated from Monkey Breast Cancer Found Infectious for Human Cells" (August 1971). In December 1971 a weekly report was prepared to clarify the premature, almost simultaneous reports of candidate human viruses made at the University of Southern California in Los Angeles and Georgetown University in Washington, D. C. (See below, "Miscellaneous Activities" for more details.) In January 1972 another weekly report noted that prostaglandins may play a role in the cancer process. In May 1972 information on cryosurgery as a technique with implications for the treatment of liver cancer was presented in a weekly report and an NIH Record story.

Reports were also written on biomedical meetings sponsored by NCI. In August 1971 a report and NIH Record story were written by a Section member on an NCI Liver Cancer Symposium in Kampala, Uganda, based on interviews with NCI scientists who had attended, and their travel reports. In September the Vth International Symposium on Comparative Leukemia Research in Padua-Venice was summarized in a weekly report. Also in September the first Airlie House meeting

of National Cancer Program Plan scientists was announced in a report. In October the Symposium on Hodgkin's Disease in St. Louis was the report topic, and in November the Symposium on Mammary Carcinoma at Cherry Hill, New Jersey. The Conference on Planning for Cancer Centers was described in a December 1971 report. In March 1972 the Breast Cancer Therapy Meeting of international scientists was reported.

In February a virology award (the Gustav Stern Award) to Dr. George J. Todaro, chief of the Viral Leukemia and Lymphoma Branch, NCI, was announced in a weekly report and NIH Record story.

In August 1971 the NCI segment of the NIH Annual Report was written by this Section, covering Long-range Planning for NCI, Viruses and Cancer, Chemicals and Cancer, Detection and Treatment, Immunology and Cancer, Molecular Biology and Cancer, and Program Administration.

NCI Research Highlights of 1971, a 27-page report on cancer research findings by NCI scientists or supported by NCI funds, was written in January 1972 by Section members. The report was divided into sections including Virus-Cancer Research, Third National Cancer Survey, Chemicals and Cancer, Cancer Detection, Cancer Drugs, Treatment with Surgery and Radiation, Immunology and Cancer.

SPEECHES, ARTICLES AND MISCELLANEOUS ACTIVITIES

In November 1971 the Section drafted additions to a speech by Secretary Richardson for the American Cancer Society's Annual Dinner. A draft was also prepared for an acceptance speech at that dinner by Representative Daniel J. Flood who received the Distinguished Service Award from ACS. Later in the same month a set of questions and answers for the Secretary's use was prepared covering such topics as Fort Detrick, virus-cancer research, and the status of Federal effort to expand national cancer research. Also in November 1971, a draft of an insert for his Committee's Report was requested by Representative Paul Rogers, and prepared by the Section.

In January 1972, a 12-page speech--"The Influence of Technology on Cancer Research"--was prepared by Section members for Surgeon General Jesse L. Steinfeld.

In January 1972, the "Blue Sheet" published detailed lists of NCI-funded Clinical Research and Program Cancer Centers. Up-to-date lists had been prepared by the Section with the help of the Program Analysis and Reporting Section for the Cancer Center Conference. (These lists have since been used extensively in reply to requests.)

In December 1971 an article on The National Cancer Act of 1971 was written with Section assistance for the Montgomery County Sentinel.

In March 1972 a six-page article was written by a Section member for the use of Secretary Richardson entitled "The Massive Attack Against Cancer" in Hadassah Magazine.

Section members arranged a filmed interview of Dr. George J. Todaro, Chief, Viral Leukemia and Lymphoma Branch, NCI, to comprise one of a series of films called "The People of Washington" sponsored by the U.S. Department of Commerce. It will be shown at Union Station when the latter becomes a Visitors Center.

Appointments were also made for several speaking engagements by NCI scientists, including one before a group of 20 French scientists visiting NIH in October.

The Section Head and staff members collaborated in developing guidelines for the announcement of research findings as a result of two premature announcements in December 1971. News conferences took place almost simultaneously--one at the University of Southern California at Los Angeles and one at Georgetown University in Washington, D. C.--that were to touch off a critical reaction from a few members of the science press. Two NCI-supported scientific teams reported their findings, prior to scientific publication, on the isolation and identification of what appeared to be human-cancer viruses. Announcements were made to the news media at press conferences despite Research Information Branch recommendations to postpone news coverage until scientific publication of their findings. After the University of Southern California conference, the Section prepared a press summary on the research there by Dr. Robert McAllister, Dr. Margery Nicholson and Dr. Robert J. Huebner--"C-Type Virus Released from Human Rhabdomyosarcoma Cells Cultured in Embryonic Kittens." In addition, a weekly report was prepared summarizing the research at both institutions and noting the impropriety of the premature announcements.

Literature Reference and Analysis

Each month more than 350 scientific journals were scanned by a Section member for publications on research findings financed by the Institute. An average of 78 references per week were recorded and a list circulated to Section writers as a source for possible news releases and weekly reports. Reference files by author, subject, and organization were maintained for background information in the writing of publications on cancer sites, current research programs, and specific information needed in answering public and media inquiries. In addition, these weekly reference lists were used by NCI's Program Analysis and Reporting Section for abstracting published papers on research supported by the Institute.

The same Section member completed the NCI portion of the NIH Scientific Directory and Annual Bibliography, an annual assignment. This publication lists the key personnel by organizational structure and all papers published by NCI scientists during the year.

Bibliographies on specific topics such as virus-cancer research, chemotherapy, Hodgkin's disease, breast cancer, asbestos in relation to cancer, insecticides, aflatoxin, and bone marrow transplants were prepared for background and in answer to inquiries. Upon request, numerous specific literature searches were made by this Section member also.

PERSONNEL

Two additional Technical Publications Writers joined the Section in May 1972, bringing the Section personnel total to eight.

Section members are encouraged to improve their skills and effectiveness through training sessions. The Section Head attended a seminar on "Current Issues in Public Information" sponsored by the U.S. Department of Agriculture Graduate School on April 5-7, 1972. Interpretation of the Freedom of Information Act was an item on the agenda.

A Section member studied organic chemistry in the Fall 1971 semester of the Graduate Program at NIH. Since September 1971 the Section's secretary has been enrolled in the NIH Upward Mobility College Program, accredited by Federal City College. Earlier, in July 1971, she attended a Better English Usage Course.

The Section Head was appointed in early 1972 to the NIH Information Training Committee.

Publications, Visuals, and Reference Section

The public information functions carried out by the Publications, Visuals, and Reference Section include the responsibility for planning, production and distribution of printed and audiovisual materials addressed to the general public as well as to professional audiences and groups within the Government. Other activities include assisting with the planning, programming, and review of Branch productions and budgets, maintaining a reference library, initiating review and approval of nonresearch materials, and screening and circulating journals and newspapers.

Publications and Other Printed Materials

Publications prepared by the Branch and other areas of the Institute were distributed to the general public, governmental public, Congressional public, health and medical groups, schools (junior high through professional) and private and voluntary organizations. Individual communications requesting printed information on cancer totaled 7,500. Over 200,000 copies of 33 publications were distributed. New publications are distributed initially to selected interest groups such as health and medical institutions, science teachers, and private and voluntary health organizations.

At the request of the White House Press Office, a packet of materials on the signing of The National Cancer Act of 1971 was sent with covering letters to approximately 2,500 science writers, science journals, DHEW Regional Medical Directors, public information officers in medical schools, and selected university and health professions administrators. The mailing included a White House

news release and fact sheet on The National Cancer Act of 1971; remarks of the President at the signing of the Act; and copies of the Act itself. Also at the request of the White House, copies of news releases announcing the appointment of the President's Cancer Panel, with a transcript of the White House press conference attended by Panel members, and the appointment of the National Cancer Advisory Board were sent to 2,500 interested individuals and groups.

As part of a new effort to communicate more fully with the scientific community (as well as other publics) NCI Cancer Center grantees were offered NCI informational materials, including publications and news releases, to help keep them abreast of NCI programs. Of 32 Centers canvassed, 24 responded affirmatively and were added to NCI's mailing list.

Arrangements were made with the Consumer Products Coordinating Center to offer the NCI publication Cancer--What to Know, What to Do About It in the quarterly issues of the Index of Consumer Production Information. To date, 50,000 copies of this NCI publication have been requested by the Center.

The publication Childhood Leukemia, A Pamphlet for Parents was reprinted for the tenth time, incorporating revisions by Dr. Edward Henderson, Chief, Hematology and Supportive Care Branch, NCI. A fact sheet on leukemia was translated into Spanish in answer to numerous requests from social service and public health workers in Spanish-speaking communities.

Lists of cancer publications available from the Institute and from the Superintendent of Documents, GPO, were revised periodically. These lists are in answer to general inquiries from individuals or groups who wish to select pertinent publications. The NCI portion of the NIH publications directory was also revised.

Steps were taken to institute procedures required by the new Departmental Publications Management Information System in the preparation of all NCI publications. With input from NCI and other areas, DHEW prints a Department Catalog of Publications designed to prevent duplication of publications by one or more agencies.

During the year the Section Head served on the NIH Liaison Committee on Printing, a committee which was established by the Office of Information, NIH, to improve administrative procedures regarding printing services, improving the quality of printing, distribution of publications, coordination with the GPO, and related matters.

A staff member provided assistance to the NCI Equal Employment Opportunity Group in the planning and production of NCI's EEO Newsletter.

In connection with the NCI Minority Conference, a conference convened to improve communications between the Institute and schools with a high percentage of minority students, information materials were mailed to prospective conferees. In addition to the regular mailing, a press release on the conference was sent to a special mailing list of Black newspapers and magazines. A display of publications with check lists for requests was exhibited at the conference, and a screening of the new NCI film, "Progress Against Cancer," was arranged.

A new effort to achieve harmony in the design of NCI publications is underway. Art work will be reviewed according to this criterion (in addition to others) whenever new publications are designed or old ones reissued. Accordingly, the redesign of some site pamphlets and planning of NCI fact sheets is underway.

The Section provided assistance to a number of Institute staff members in the design, production and distribution of publications and other printed materials.

Audiovisuals

Existing films, slides, and visual materials were supplied upon request to NCI staff, other Government agencies, the media, and the public. New photographs, charts, slides, and other audiovisual materials were prepared on a continuing basis. Assistance was provided to the media in obtaining or making photographs and films.

The National Cancer Institute was awarded the Silver Certificate of Merit by the National Medical Association, Inc., for an exhibit titled "Research and Related Programs of the National Cancer Institute." The exhibit was shown at the annual meeting of the NMA, one of nine showings arranged during the year. The exhibit is now being redesigned for showing at the 7th National Cancer Conference to be held in Los Angeles in September 1972.

The NCI exhibit "Cancer Publications and Journals" is also being up-dated for the same Conference.

Six new displays were prepared during the year for conferences and bulletin boards.

The Section Head assisted the Acting Chief, Research Information Branch, in arranging several screenings of the new film "Progress Against Cancer" prior to its release for network television. Plans are underway to cut the 58-1/2-minute film to a 27-1/2-minute version for wide distribution by a film distributer. It is planned that at least 200 prints be ordered for this purpose.

The Section Head, with the Acting Branch Chief, screened a film made when The National Cancer Act of 1971 was signed by President Nixon. Consideration was given to future use of clips from this film.

The Section handled requests from the printed and electronic news media and arranged for television and radio appearances of NCI staff. Assistance was provided to the NIH Information Office in preparing radio and TV spots.

A Section member assisted the Chief, Research Information Branch, with arrangements for the visit of Prince Hitachi of Japan in preparing the lobby of Building 37 and arranging photographs of the Prince and Princess, attendants, and NIH officials.

Other Activities

The Section Head attended a week-long NIH supervisory training course required of supervisors and attended a 3-day EEO Conference of NCI Section Heads held in Annapolis, Maryland.

A Section member attended a 40-hour course, "Effective Writing," sponsored by the NIH Personnel Training Office.

At the request of the HEW Under Secretary for Public Affairs, via NIH Information Office, the Section prepared six 5" x 8" cards summarizing major NCI program areas for use by senior staff level Departmental officials in making speeches. These cards will be brought up to date periodically as directed by HEW.

The Section prepared text and source material for some charts used in Volume II, Part I, The Strategic Plan, National Cancer Program Plan.

Over 30 news releases and summaries were mailed to the news media.

NCI professional staff members and grantees were recruited by Section staff members to appear as speakers at meetings in various locations.

A temporary employee was assigned to the Section for several weeks and produced a brief report to appear in an article in Military Medicine.

A Section member received a merit award for outstanding service during the year.

Reference Service

The daily screening of newspapers, adding clippings of particular NCI interest to the NIH "Current Clips," became of increasing value to NCI staff. Activity by the Congress on cancer legislation stimulated extensive coverage of the subject by the print media. The "Clips" and addenda were distributed to senior NCI staff members during the year.

Medical news magazines and other printed materials and periodicals received in the Branch were also screened for articles on cancer, and those of interest to Branch staff were circulated. These materials and other reference items are retained in the Section reference library for use by Institute staff.

Review and Approval of Nonresearch Materials

Approximately 150 nonresearch items prepared by the Institute staff for publication or other official use were reviewed and processed in the Section, forwarded to the Branch Chief for approval, and thence to other channels required by NIH Policy and procedure directives.

Assistance was given to Institute staff in initiating clearance of materials. With the advent of new HEW requirements for placing numbers on publications, all NCI publications now are processed by the Section.

Monthly reports of materials cleared and in clearance were prepared and appended to the JNCI Board of Editors' reports of research materials in clearance.

RESEARCH CONTRACTS BRANCH

Introduction

Any organization as active as is NCI in the award of research contracts is in need of a highly experienced staff of professional contract negotiators and supporting contract administrative personnel. This staff must also have the ability to work closely and knowledgeably with scientific project officers in order to insure that the research contract is a suitable instrument for the attainment of research objectives. The Yarborough Commission, GAO and the Departmental Committee which reported on NIH Contract Management (the Sisco Committee) have recognized the importance of contract negotiations to NCI's mission, and their recommendations have resulted in sweeping changes in NCI contract management during the last year, which have had the effect of elevating the organizational stature of this function. These changes have also been a response to the dramatic growth in NCI contracting.

The following table depicts the growth in the past three years of the contracts activity. It can be seen that the number of contracts in force at NCI has grown from 333 to 627 in the period of FY 1970 to FY 1972, while the dollar amounts have grown from $49,704,000 to $128,143,000. All scientific areas have placed increased reliance upon contracts, but the biggest growth has been in Etiology, from $26,826,000 to $70,685,000, and within Etiology the carcinogenesis area has grown the most rapidly. This kind of growth in reliance upon contracts must necessarily be a reflection of the proven soundness of the contract instrument as a method for achieving results in cancer research.

RESEARCH CONTRACTS AWARDED

NCI, BY DOLLARS AND NUMBERS

Scientific Program Area	1970	1971	1972
	(Dollars in thousands) (Years are Fiscal Years)		
Etiology			
Carcinogenesis	$ 7,075	$10,573	$28,947
(Numbers)	(41)	(71)	(110)
Viral Oncology	17,739	31,411	35,976
(Numbers)	(93)	(117)	(136)
Demography	2,012	4,412	5,762
(Numbers)	(37)	(51)	(55)
Sub-total, Etiology	26,826	46,396	70,685
(Numbers)	(171)	(239)	(301)
Chemotherapy	19,677	24,235	41,826
(Numbers)	(125)	(165)	(230)
General Laboratories & Clinics	3,201	4,354	9,622
(Numbers)	(37)	(40)	(93)
Major Construction and Other	-	3,000	6,000
(Numbers)	-	(1)	(3)
Totals	$49,704	$77,985	$128,143
(Numbers)	(333)	(445)	(627)

Note: Data for 1972 estimated

RESEARCH CONTRACTS BRANCH

Administration of the Contract Program

During the past year, the following administrative changes have been made in contracts:

1. The NCI (along with other major NIH Institutes) has been delegated full authority to contract.

2. The Research Contracts Branch of NIH, which previously had possessed the contracting authority for all the Institutes of NIH, has been assigned staff responsibilities and many of its personnel have been transferred to NCI, as well as to the other major Institutes.

3. The former Grants and Research Contracts Operations Branch, which had been located in Extramural Activities, NCI, has been divested of its grants operations responsibilities and has been transferred to the Office of the Director and renamed the Research Contracts Branch. Its staff has been augmented by transfers of contracts specialists from the scientific program areas, the transferees from NIH already mentioned, and recruitments from outside NIH.

4. This new Research Contracts Branch, OD, NCI, is now headed by Mr. Carl Fretts, who is also the Chief Contracting Officer. His staff of 72 personnel is decentralized and located adjacent to the Scientific Program Areas, in order to provide maximum support to these programs. Each of the three Scientific Program Areas has had assigned to it a contracting officer to head the contracts staff working with it.

Owing to the foregoing measures, the disadvantages of the formerly fragmented system of contract management have been overcome, with consequent advantage to the scientific program areas in terms of more responsive contract administration.

At year end, studies were under way to ascertain what further authorities to contract accrue to NCI pursuant to the National Cancer Act of 1971.

NATIONAL CANCER INSTITUTE
CHEMOTHERAPY

Annual Report of the Scientific Director
July 1, 1971 - June 30, 1972

Introduction

The objective of the Chemotherapy program is the chemical control of cancer. Most of the deaths from cancer occur because of failure of the localized strategy for cure, namely, surgery or irradiation. The major hope for control of disseminated cancer in the near future lies in control by means of drugs. Since 1954 the National Cancer Institute has been developing the means by which this objective could be achieved. The core of this activity has been the establishment of a central focal point that would provide leadership of the national effort and would take all those steps necessary to achieve control of cancer through the use of drugs.

This leadership role is carried out by a scientific team consisting of NCI staff and a number of distinguished scientists and physicians from similar research institutes and from the universities. The conceptual and theoretical aspects of drug control of cancer are developed by active discussions and cooperative studies between these outside advisory members of the team and scientists at NCI. The management of the continuing activities necessary to the implementation of the Chemotherapy program is carried out by the NCI staff.

The Scientific Team

The activities of this scientific team can be categorized under five headings.

1. It carries out research in the laboratories and clinics in Bethesda and in other research centers.

2. The team is responsible for a total program of drug development. United States pharmaceutical houses do not have extensive programs for anti-cancer drugs and the government has established its own drug development activities in order that a ready supply of effective new agents would be available for clinical usage. While this is done mostly under contract, the complete plan for drug development has been worked out by the team. The NCI members of the team are responsible for its management and coordination.

3. The team serves as a national clearing house and operations center for much of the work in chemotherapy that is going on in this country. The activities consist of collection of information on chemical control of cancer, analysis of the data and distribution to interested parties. The team provides drugs, radioactively labeled materials, and help of all sorts to the national cooperative clinical groups as well as to scientists in laboratories.

4. The team carries out a worldwide surveillance for leads on new drugs or better ways of using them. Not only are ideas and drugs exchanged, but laboratory and clinical information from other research centers around the world is assembled, analyzed, and distributed.

5. Since most initial leads on active drugs in cancer come from empiric observations, an important contribution of the team is the illumination of these empiric successes by all the light provided by modern science. In this way empiric successes can be generalized into sound theory that then becomes a much more precise tool in predicting drug effects in man from animal and in vitro observation.

Criteria for Judging Success of the Chemotherapy Program

What criteria should be used to measure the success of the Chemotherapy program? There is a single major criterion: the achievement of normal life expectancy in patients who would have died from cancer. There are two minor criteria; short-term endpoints that we know from experience are milestones on the way to achievement of normal life expectancy. The first of these is an increasing percentage in the number of patients with disseminated cancer who can be put into complete remission of their disease by means of drugs. The second such milestone is the gradual lengthening of disease free remission. For example, 15 years ago patients with acute lymphocytic leukemia achieved complete remissio after drugs in 20% of instances and had a disease free remission of less than six months. As improvements were made in gaining chemical control the complete remission rate rose to 95% + and the duration of remission gradually crept up to a year, then two years, then three years, and so on.

In applying the major criterion to the program, it is quite clear that normal life expectancy has already been demonstrated through the use of drugs in 10 kinds of clinical cancer. These include acute lymphocytic leukemia, Hodgkin's disease, Burkitt's lymphoma, lymphosarcoma, reticulum cell sarcoma, choriocarcinoma, Ewing's tumor, Wilms' tumor, testicular tumors and retinoblastoma. In terms of the minor criteria, it is clear that complete remissions and increasing durations of remission are occurring in acute myelocytic leukemia, breast cancer, oat cell cancer of the lung, rhabdomyosarcoma, Kaposi's sarcoma, and osteogenic sarcoma. It will take many years before one can establish normal life expectancy from these current studies.

The tumors that are highly susceptible to drug constitute about 15% of clinical cancer. These cancers have biological similarities in that all are rapidly growing, whereas the tumors that are not so susceptible to drugs grow much more slowly. Extensive research into the reasons for this has shown that rapid growt and drug susceptibility are both linked to the percentage of cells that are in DNA synthesis at any one time. Conversely, the slowly growing, drug insensitive tumors have few cells in DNA synthesis. This theoretical basis for drug sensitivity has led to many new insights on the chemical control of slowly growing tumors.

The Linear Array

When the Cancer Institute in 1965 reviewed the results of the first ten years of the operation of this program, it was possible on the basis of the informatio

then available to set up what is called a linear array. This consists of all the logical steps that should be taken in examining the chemical universe and seeing whether new materials can be found that could control cancer in a better way than current drugs. This goes all the way from acquisition of new materials through the final definitive clinical trial and introduction of the active materials into medical practice.

The linear array is by no means a static affair, but is continually changing in its details, although not in its overall format, as experience and new knowledge teach that there are better ways of operating. Its usefulness consists in that first it provides the logic for telling us whether a new drug should move ahead toward clinical trial, or should be dropped, or recycled to get information on some important sidelight that developed during its initial study. It allows development of a decision network in which it is possible to spell out sharply the criteria that will be used for moving from one step to another. It can be used as an operating blueprint and the scientific team, over the years, had developed a facility in using this blueprint in its day to day operation. It is also highly useful for planning purposes. For example, in trying to plan ahead for resources, it will be of great usefulness in coordinating the National Plan with the ongoing activities within the Chemotherapy program. Finally, the linear array provides a means of achieving an optimal balance among various elements of the program, so that resources at every stage of development will be ready for the volume of work required.

Key Issues and Plans

The major task of the Chemotherapy program is the continuing responsibility for drug development. This means taking drugs all the way from acquisition of new materials through screening, toxicology, pharmacology, large scale production, formulation, FDA licensure, safety studies in man, and definitive clinical trial. The drugs now under clinical trial began their way through the system three to nine years ago and are appearing in the clinic at present at the rate of seven to eight new drugs per year. In order to increase the number of active compounds in the future, the input of new materials to the screen is being raised from 15,000 to 20,000 per annum. In addition, the program is concentrating on several problems, the solution of which will extend these gains to many more patients.

1. Cell Population Kinetics. Because drug control is limited to a particular kind of tumor biology, namely, rapidly growing tumors, there is a broad effort to understand - biologically, clinically and in a molecular sense - the reasons for lack of drug susceptibility of slowly growing tumors. Additional screens, simulating the biology of slowly growing tumors, are being added.

2. Pharmacokinetics. The other major reason for lack of drug response is failure of the drug to reach its target in sufficient concentrations to kill the tumor cells, and, therefore, there is intensive work ongoing on the pharmacologic disposition of anti-tumor drugs. New methodologies have made it possible to study the pharmacology of many older drugs, whose disposition is not fully understood. Plans are underway to increase pharmacologic resources so that each new drug will be completely studied before it reaches the clinic. Special emphasis continues to be placed upon the difficulties of getting drugs into tumors within the central nervous system.

3. Assay of Tumor Cells. Another major obstacle to the cure of cancer is the inability of the clinician to know whether treatment has rid the body of all tumor cells. Clinically diagnosable cancer is associated with a body burden of approximately 1×10^{12} tumor cells. When treatment reduces the number to 1×10^{9} cells, the tumor is no longer detectable yet will always return. Hence treatment is often too short or lacks sufficient intensity to bring about cure. While it is true that single modalities of treatment - such as surgery or a single drug - cannot effect the required reduction, combination therapy may well be adequate. In order to assess rapidly the curative capacity of combination therapy, a method of calculation of the number of tumor cells in the body after each course of therapy is essential. Research in the past year by Dr. Sydney Salmon has developed a capability of such calculation in multiple myeloma. The principles, however, may possibly be valid in a variety of cancer and this application is under active pursuit.

4. Supportive Care to Infections. Another obstacle to curative therapy is the morbidity and mortality from infection, resulting from the side effects of treatment. Successes in the prevention and treatment of infections by means of protected environments and white cell transfusions have led to expanded utilization of these techniques.

5. Phase II Studies. The biggest lag in evaluation of combination therapy is the result of inadequate clinical resources for Phase II (therapeutic effectiveness) studies. Extensive efforts for expansion are underway, both through contracts and by increasingly active liaison with grantees. In the rapidly growing tumors, Phase II studies are proceeding in the acute leukemias, lymphomas, melanoma, and brain tumors. This year will see addition of osteogenic sarcoma, ovarian cancer, and testicular cancer. In the slowly growing tumors, cancers of the breast, lung, and colon are the main targets, but tumors of the pancreas and liver will be added.

6. Biochemical and Other In Vitro Studies. The rapid advance in knowledge during the past year concerning reverse transcriptase in animal tumors and in the acute leukemias has led to the expansion of these studies with emphasis on drug inhibitors of this key enzyme. The recent findings in the delineation of the various polymerases and their role in molecular control of growth has resulted in a joint Etiology-Chemotherapy effort to emphasize development of this area.

7. Immunotherapy. The most promising clinical application of immunotherapy for the control of cancer resides in the enhancement of nonspecific resistance to tumors brought about by vaccination with BCG strain of tubercle bacillus. Recent advances in this will be applied in clinical studies of acute leukemia and melanoma.

Conclusion

From the operation of this program over the last 15 years, there has come a sound body of data on the chemical control of clinical cancer and a precise knowledge of the confidence that can be placed in the animal systems in predicting what will happen in man, both with respect to effects upon the tumor and effects upon the patient. There are now about 40 drugs that have been

shown to cause regression of some type of clinical cancer. There are at least 100 different kinds of cancer. But of all these, only two, choriocarcinoma and Burkitt's lymphoma, can be cured by the use of a single drug. In all other instances, either a combination of drugs is required or else drugs must be in association with surgery and radiation to kill those tumor cells that have been left behind by these localized forms of cancer therapy. The intensive use of combinations of drugs or drugs plus surgery and radiation has changed the whole outlook of patients with presumably fatal cancer. While certainly the scientific team must continue its activities in drug development, the real challenge is the best way to combine known effective ways of treating cancer to the optimal benefit of each patient.

CHEMOTHERAPY LINEAR ARRAY CLASSIFICATION CODE

Stage	Code	Description of Activity
I	1XX	Acquisition of new agents (includes studies of cellular control mechanisms).
IIA	2AX	Determination of anti-tumor activity of new agents.
IIB	2B1	Develop acceptable experimental formulation.
IIB	2B2	Detailed evaluation of new agents - dose, route, and schedule dependency (in vivo and in vitro systems). Cell kinetics.
IIB	2B3	Initial animal pharmacology studies; evaluation for absorption and anti-tumor activity after oral administration.
IIB	2B4	Procurement of sufficient amounts of new agent for preclinical studies.
III	3X1	Toxicology and pharmacology in large animals (dogs and monkeys).
III	3X2	Production and formulation for clinical trials.
IV	4X1	Clinical Trials* - Phase I. Determination of tolerated dose. Cell kinetics.
IV	4X2	Clinical pharmacology - initial pharmacologic studies in man associated with early clinical trials.
V	5XX	Clinical Trials* - Phase II. Initial therapeutic trials. Development of "markers".
VI	6X1	Clinical Trials* - Phase III. Initial eradicative trials in man. Agent used singly or in combination with other agents in comparative study . Research, development and subsequent evaluation in supportive care (protected envirnment, platelet and leukocyte transfusions, antibiotics, etc.)
VI	6X2	Toxicologic and pharmacologic studies in large animals and man associated with further development of a drug.
VII	7XX	Clinical Trials* - adjuvant studies (radiotherapy, surgery, immunotherapy). Determination of role of agent in total care.
---	8XX	Unassigned. Includes administration, dissemination of information to medical and scientific community.

* Supportive care used as ancillary therapy is prorated among the phases of clinical trials using such resources.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES IN

THE CHEMOTHERAPY PROGRAM

TYPE OF ACTIVITY BY LINEAR ARRAY CLASSIFICATION		NO. OF CONTRACTS	ANNUAL LEVEL[1/]	(PERCENT)
TOTAL		188[2/]	$36,715,367	
STAGE I			7,084,176	(19.3)
1XX	Acquisition of New Agents	98	7,084,176	(19.3)
STAGE IIA			10,633,130	(29.0)
2AX	Determination of Anti-tumor Activity	67	10,633,130	(29.0)
STAGE IIB			5,514,383	(15.0)
2B1	Experimental Formulation Development	4	267,128	(0.7)
2B2	Detailed Evaluation of New Agents	56	3,789,796	(10.3)
2B3	Initial Animal Pharm. Studies	2	70,000	(0.2)
2B4	Procure. of Agents for Preclinical Studies	13	1,387,459	(3.8)
STAGE III			5,108,489	(13.9)
3X1	Toxicology and Pharm. in Large Animals	15	1,853,285	(5.0)
3X2	Product. and Formulation for Clinical Trials	11	3,255,204	(8.9)
STAGE IV			2,230,805	(6.1)
4X1	Clinical Trials - Phase I	4	589,150	(1.6)
4X2	Initial Clinical Pharm. Studies in Man	9	1,641,655	(4.5)
STAGE V			651,547	(1.8)
5XX	Clinical Trials - Phase II	8	651,547	(1.8)
STAGE VI			4,387,420	(11.9)
6X1	Clinical Trials - Phase III	18	1,914,135	(5.2)
6X2	Tox. & Pharm. Studies in Large Anim. & Man	25	2,473,285	(6.7)
STAGE VII			1,037,200	(2.8)
7XX	Clinical Trials - Adjuvant Studies	6	1,037,200	(2.8)
UNASSIGNED			68,217	(0.2)
8XX	Administration and Information Functions	4	68,217	(0.2)

1/ Funds obligated during Fiscal Year 1972. Not included are funds utilized for direct purchase of clinical drugs (approximately $1,403,483) and about twenty-seven new contracts not negotiated as of this report ($2,519,000).

2/ Since many contracts involve more than one activity, the totals of Stages I through VII and the unassigned category do not correspond with the total number of contracts.

TABLE II

Analysis of Contracts

By Activity

ACTIVITY / CONTRACT	DOLLAR LEVEL[1/]	
Stage I - Acquisition of New Agents		$7,084,176
Agriculture, Dept. of (64-1)	307,400	
Arizona State Univ. (71-2308)	45,000	
Arizona, Univ. of (67-1484)	80,000	
ARS/Sprague-Dawley (71-2342)	12,600	
ARS/Sprague-Dawley (72-359)	24,898	
ARS/Sprague-Dawley (71-2007)	85,573	
ARS/Sprague-Dawley (70-2044)	16,800	
Ash Stevens, Inc. (71-2314)	68,000	
Assoc. Biomedic Systems, Inc.(70-2271)	18,950	
Battelle Memorial Inst. (70-2008)	10,008	
Battelle Memorial Inst. (71-2182)	69,000	
Battelle Memorial Inst. (69-2251)	24,500	
Bellaire Acres (71-652)	3,265	
Bellaire Acres (72-376)	29,484	
Bellaire Acres (72-729)	7,862	
Bionetics Res. Lab. (71-2340)	196,500	
Bristol Myers Co. (69-35)	605,000	
Chas. Riv. Breeding (70-2043)	13,790	
Chas. Riv. Breeding (72-377)	30,849	
Chas. Riv. Breeding (72-730)	7,790	
Chas. Riv. Breeding (72-2004)	86,961	
Chas. Riv. Breeding (71-2343)	12,600	
Chemical Abstract Svc. (65-1021)	104,499	
Collaborative Res., Inc. (66-506)	19,720	
Collaborative Res., Inc. (71-2326)	50,000	
Colorado, Univ. of (71-2186)	37,500	
Cornell Univ. Med. Col. (69-60)	39,900	
Costa Rica, Univ. of (64-31)	700	
Delaware, Univ. of (69-2115)	18,791	
Dow Chemical Co. (71-2313)	90,176	
Flow Res. Anim., Inc. (72-378)	22,714	
Georgia, Inst. of Tech. (68-1288)	15,000	
Georgia, Univ. of (71-2311)	34,900	
Germfree Life Res. Center (72-3204)	22,911	
Harlan Industries (72-419)	24,242	
Harlan Industries (72-724)	8,154	
Harlan Industries (72-3218)	16,629	
Hawaii, Univ. of (67-748)	46,000	
Horton Labs. (72-361)	8,445	

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage I - Acquisition of New Agents (continued)	
ITT Research Inst. (68-1021)	100,000
Illinois, Univ. of (72-2078)	86,000
Illinois, Univ. of (72-3208)	55,375
Inst. Jules Bordet (72-2053)	7,200
Jackson Lab. (71-1004)	41,199
Japanese Fdn. for Cancer Res.(72-2054)	20,174
Johns Hopkins University (72-3224)	37,500
Kansas, Univ. of (71-2010)	10,874
Lab. Supply Co. (72-360)	24,897
Lab. Supply Co. (70-2041)	16,547
Lab. Supply Co. (72-733)	8,300
Little, A.D. (65-61)	50,000
Little, A.D. (72-3257)	16,000
Mason Research Inst. (71-2259)	5,600
Miami, Univ. of (70-2225)	32,132
Michigan Dept. of Health (65-7)	42,655
Michigan, Univ. of (69-2004)	47,866
Microbiological Assoc., Inc. (68-1283)	600,000
Midwest Res. Inst. (65-94)	291,308
Nat. Acad. of Sciences (64-44)(TO#6)	2,450
Nat. Acad. of Sciences (64-44)(TO#16)	2,800
N.J. College of Med. & Dent. (72-3211)	22,400
N.Y. State Dept. of Health (70-2035)	10,494
Pennsylvania, Univ. of (71-2187)	150,000
Pennwalt Corp. (71-2309)	59,890
Pomona College (69-2112)	37,251
Rawley Farms (72-380)	28,938
Research Triangle Inst. (69-2019)	100,000
Rhode Island Univ. (71-2312)	39,778
Scripps Clinic and Res. Fdn. (72-2042)	19,000
Sheehan Inst. for Res. (71-2207)	45,000
Simonsen Labs. (72-3219)	17,131
Simonsen Labs. (72-409)	24,680
Simonsen Labs. (71-657)	1,736
Simonsen Labs. (71-2101)	25,110
Simonsen Labs. (72-725)	8,445
Southern Animal Farms (72-732)	8,590
Southern Animal Farms (72-379)	23,150

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage I - Acquisition of New Agents (continued)	
Southern Animal Farms (72-2013)	17,036
Southern Res. Inst. (71-2021)	456,000
Southern Res. Inst. (66-29)	250,000
Southern Res. Inst. (71-2098)	100,000
Stanford Research Inst. (71-2070)	400,000
Stanford Research Inst. (69-37)	27,425
Starks Assoc., Inc. (68-1275)	46,050
Taconic Farms (72-381)	18,564
Texas Inbred Mice (68-88)	60,488
Texas Inbred Mice (72-375)	20,382
TRW, Inc. (63-622)	219,000
Tulane University (65-593)	17,120
Upjohn Co. (68-1023)	207,000
Utah, Univ. of (65-1041)	120,000
Virginia, Univ. of (71-2099)	60,920
Vitro Laboratories (69-58)	96,417
Warf Institute, Inc. (66-494)	392,000
Warf Institute, Inc. (67 747)	18,583
Washington State Univ. (72-2059)	20,000
Wisconsin, Univ. of (67-1126)	15,995
Wisconsin, Univ. of (71-2275)	83,615
Stage IIA - Determination of Anti-Tumor Activity of New Agents	
	$10,633,130
ARS/Sprague-Dawley (72-359)	112,039
ARS/Sprague-Dawley (70-2044)	75,600
ARS/Sprague-Dawley (71-2007)	385,079
ARS/Sprague-Dawley (71-2342)	56,700
Atomic Energy Commission (71-56)	10,000
Battelle Memorial Inst. (70-2008)	45,036
Battelle Memorial Inst. (71-2182)	280,600
Battelle Memorial Inst. (69-2251)	110,250
Bellaire Acres (72-376)	132,678
Bellaire Acres (71-652)	14,685
Bellaire Acres (72-729)	35,381

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage IIA - Determination of Anti-Tumor Activity of New Agents (continued)	
Bristol- Myers Co. (69-35)	80,000
Chas. Riv. Breeding (72-377)	138,820
Chas. Riv. Breeding (70-2043)	62,055
Chas. Riv. Breeding (72-730)	35,053
Chas. Riv. Breeding (72-2004)	436,322
Chas. Riv. Breeding (71-668)	46,875
Chas. Riv. Breeding (71-2343)	56,700
Flow Res. Animal, Inc. (72-378)	102,211
Germfree Life Res. Center (72-3204)	103,099
Harlan Industries (72-419)	109,091
Harlan Industries (72-3218)	74,831
Harlan Industries (71-646)	46,875
Harlan Industries (72-724)	36,691
Horton Labs (72-361)	38,002
Horton Labs (71-709)	46,875
IIT Research Inst. (68-1021)	1,233,000
Jackson Lab. (71-1004)	185,396
Kansas, Univ. of (71-2010)	48,933
Lab. Supply Co. (72-360)	112,040
Lab. Supply Co. (70-2041)	74,460
Lab. Supply Co. (72-733)	37,346
Little, A.D. (65-61)	800,000
Little, A.D. (72-3257)	79,500
Mason Res. Inst. (71-2259)	489,560
Miami, Univ. of (70-2225)	48,000
Micro. Assoc. Inc. (68-1283)	650,000
Nat. Acad. of Sciences (64-44)(TO#6)	11,025
Nat. Acad. of Sciences (64-44)(TO#16)	12,600
N.J. College of Med. & Dent. (66-52)	7,330
N.J. College of Med. & Dent. (72-3211)	100,800
N.Y. State Dept. of Health (70-2035)	47,220
Pennsylvania, Univ. of (71-2188)	10,550
Rawley Farms (72-380)	130,221
Scripps Clinic & Res. Fdn. (72-2042)	20,000
Simonsen Labs (72-409)	111,056
Simonsen Labs (72-3219)	77,092
Simonsen Labs (71-657)	7,812
Simonsen Labs (71-2101)	112,993

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage IIA - Determination of Anti-Tumor Activity of New Agents (continued)	
Simonsen Labs (72-725)	38,000
Southern Animal Farms (72-379)	104,177
Southern Animal Farms (72-732)	38,657
Southern Animal Farms (71-645)	46,875
Southern Animal Farms (72-2013)	76,660
Southern Res. Inst. (71-2189)	27,400
Southern Res. Inst. (71-2098)	1,225,000
Taconic Farms (72-381)	83,538
Texas Inbred Mice (72-375)	91,730
Texas Inbred Mice (68-88)	272,196
TRW, Inc. (63-622)	500,000
Tulane University (65-593)	77,042
Upjohn Company (68-1023)	190,000
Vitro Laboratories (69-58)	403,747
Warf Inst., Inc. (66-494)	231,000
Washington State Univ. (72-2059)	83,626
Yale University (67-1193)	10,000
Yale University (71-2339)	55,000
Stage IIB - Develop Acceptable Experimental Formulation	$267,128
Ben Venue Labs., Inc. (66-909)	72,200
Michigan, Univ. of (68-1284)	70,000
Midwest Res. Inst. (69-2113)	100,000
Phillips Roxane Labs. (71-2139)	24,928
Stage IIB - Detailed Evaluation of New Agents	$3,789,796
ARS/Sprague-Dawley (70-2044)	27,600
ARS/Sprague-Dawley (71-2342)	20,700
ARS/Sprague-Dawley (72-359)	40,903
ARS/Sprague-Dawley (71-2007)	140,585
Atomic Energy Commission (71-56)	9,000
Battelle Memorial Inst. (70-2008)	16,441
Battelle Memorial Inst. (71-2182)	110,400

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage IIB - Detailed Evaluation of New Agents (continued)	
Battelle Memorial Inst. (69-2251)	40,250
Bellaire Acres (71-652)	5,360
Bellaire Acres (72-376)	48,438
Bellaire Acres (72-729)	12,917
Bristol-Myers Company (69-35)	10,000
Chas. Riv. Breeding (72-730)	12,797
Chas. Riv. Breeding (70-2043)	22,655
Chas, Riv. Breeding (71-2343)	20,700
Chas. Riv. Breeding (72-377)	50,681
Chas. Riv. Breeding (72-2004)	159,286
Flow Res. Anim. Inc. (72-378)	37,315
Germfree Life Res. Center (72-3204)	37,639
Harlan Industries (72-419)	39,827
Harlan Industries (72-724)	13,395
Harlan Industries (72-3218)	27,319
Horton Labs. (72-361)	13,873
IIT Research Inst. (68-1021)	106,000
Jackson Lab. (71-1004)	67,684
Kansas, Univ. of (71-2010)	17,864
Lab. Supply Co. (72-360)	40,903
Lab. Supply Co. (70-2041)	27,184
Lab. Supply Co. (72-733)	13,634
Little, A.D. (65-61)	400,000
Little, A.D. (72-3257)	23,700
Mason Research Institute (71-2259)	64,840
Micro. Assoc. (68-1283)	450,000
Nat. Acad. of Sciences (64-44)(TO#6&16)	8,625
N.J. College of Med. & Dent. (66-52)	2,440
N.J. College of Med. & Dent. (72-3211)	36,800
N.Y. State Dept. of Health (70-2035)	17,239
Rawley Farms (72-380)	47,541
Simonsen Labs (72-3219)	28,144
Simonsen Labs (72-409)	40,544
Simonsen Labs (72-725)	13,875
Simonsen Labs (71-2101)	41,251
Simonsen Labs (71-657)	2,852
Southern Animal Farms (72-379)	38,033

TABLE II

(continued)

ACTIVITY / CONTRACTS	DOLLAR LEVEL[1/]
Stage III - Toxicology and Pharmacology in Large Animals	**$1,853,285**
Antec Corp. (71-380)	13,575
Battelle Memorial Inst. (71-2262)	56,255
Hazelton Res. Anim. (72-410)	11,775
IIT Research Inst. (67-1141)	149,294
Int'l Res. & Dev. Corp. (71-2264)	47,658
Little, A.D. (65-61)	300,000
Marshall Res. Anim. (71-378)	15,225
Mason Res. Inst. (70-2055)	423,118
Microbiological Assoc., Inc. (68-1283)	150,000
Midwest Res. Inst. (71-2263)	51,813
Monsanto Research Corp. (65-1044)	50,000
South Shore Anal. & Res. Lab. (64-932)	179,994
Southern Research Inst. (70-2083)	217,500
TRW, Inc. (69-2079)	33,320
Yale University (70-2089)	153,758
Stage III - Production and Formulation for Clinical Trials	**$3,255,204**
Aerojet-Gen. Corp. (68-1300)	123,000
Agriculture, Dept. of (64-1)	14,000
Ben Venue Labs., Inc. (66-909)	650,357
Bristol-Myers Co. (69-35)	22,000
Dow Chemical Co. (71-2165)	60,000
Merck & Co. (72-2002)	54,000
Midwest Res. Inst. (69-2113)	59,000
Monsanto Res. Corp. (67-658)	120,000
Philips Roxane Labs. (71-2139)	113,562
Stanford Res. Inst. (71-2070)	78,000
Starks Assoc. Inc. (62-484)	108,000
Stage IV - Clinical Trials - Phase I, Determination of Tolerated Dose	**$589,150**
Atomic Energy Comm. (71-59)	36,400
Duke Univ. Med. Cntr. (72-2044)	93,300

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage IIB - Detailed Evaluation of New Agents (continued)	
Southern Animal Farms (72-732)	14,113
Southern Animal Farms (72-2013)	27,987
Southern Res. Inst. (66-29)	252,000
Southern Res. Inst. (71-2098)	443,000
Taconic Farms (72-381)	30,498
Texas Inbred Mice (68-88)	99,374
Texas Inbred Mice (72-375)	33,488
TRW, Inc. (63-622)	250,000
Tulane University (65-593)	28,128
Vitro Laboratories (69-58)	102,444
Warf Inst., Inc. (66-494)	71,000
Washington State Univ. (72-2059)	30,530
Stage IIB - Evaluation for Absorption and Anti-Tumor Activity After Oral Administration	$70,000
Microbiological Assoc. (68-1283)	30,000
Stanford Res. Institute (71-2162)	40,000
Stage IIB - Procurement of Sufficient Amounts of New Agents for Pre-Clinical Studies	$1,387,459
Aerojet-General Corp. (68-1300)	100,000
Agriculture, Dept. of (64-1)	16,000
Collaborative Res., Inc. (66-506)	62,455
Dow Chemical Co. (71-2165)	90,986
Hawaii, Univ. of (67-748)	45,000
Merck & Co. (72-2002)	453,000
Monsanto Res. Corp. (67-658)	38,000
Research Triangle Inst. (69-2019)	39,968
Stanford Research Inst. (71-2070)	150,000
Starks Assoc., Inc. (62-484)	108,000
Starks Assoc., Inc. (68-1275)	46,050
Starks Assoc., Inc. (72-3203)	204,000
Virginia, Univ. of (71-2099)	34,000

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage IV - Clinical Trials - Phase I, Determination of Tolerated Dose (continued)	
National Bur. of Standards (69-49)	161,000
TRW, Inc. (68-1286)	298,450
Stage IV - Clinical Pharmacology	$1,641,655
Jefferson Med. Col (70-2136)	50,000
Little, A.D. (65-61)	700,000
Microbiological Assoc. Inc. (68-1283)	330,000
Monsanto Res. Corp. (65-1044)	25,000
Southern Res. Inst. (65-654)	170,975
Stanford Res. Inst. (71-2162)	38,680
Texas, Univ. of (66-1156)	132,000
Upjohn Co. (68-1023)	190,000
Yale Univ. (67-1193)	5,000
Stage V - Clinical Trials - Phase II, Initial Therapeutic Trials	$651,547
Albany Med. Col. (72-2074)	49,736
Makerere Univ. (67-1343)	57,500
Mayo Foundation (71-2185)	98,800
Mayo Foundation (70-2066)	100,000
Memorial Hospital (72-2073)	192,627
N.Y. State Dept. of Health (72-2072)	78,800
Vitro Labs (72-2021)	24,735
Walter Reed Army Med. Cntr. (64-5)	49,349
Stage VI - Clinical Trials - Phase III, Initial Eradicative Trials in Man	$1,914,135
Abcor, Inc. (70-2239)	4,000
American Red Cross (70-2005)	110,284
Biolabs, Inc. (72-2007)	9,091
Bowman Gray Sch. of Med. (68-950)	18,262

TABLE II

(continued)

ACTIVITY CONTRACTS	DOLLAR LEVEL[1/]
Stage VI - Clinical Trials - Phase III, Initial Eradicative Trials in Man (continued)	
California, Univ. of (70-2014)	36,968
California, Univ. of (70-2092)	117,000
Connecticut, Univ. of (71-2181)	33,980
Duke University (70-2030)	51,218
Georgetown Univ. (71-2123)	287,470
Indiana Univ. Fdn. (70-2028)	18,000
Kentucky, Univ. of (67-1173)	32,517
Makerere Univ. (67-1343)	96,250
Microbiological Assoc., Inc. (69-2177)	135,000
New York Univ. (67-1174)	25,300
Ohio State Univ. (67-1175)	25,060
Texas, Univ. of (71-2094)	425,000
Veterans Adm. (64-4)	464,000
Vitro Labs (72-2021)	24,735
Stage VI - Toxicologic and Pharmacologic Studies in Large Animals and Man	$2,473,285
Assoc. Biomedic Systems, Inc. (70-2271)	75,800
Atomic Energy Comm. (71-60)	60,000
Bionetics Res. Lab. (72-2062)	87,216
Bionetics Res. Lab. (71-2340)	589,400
Brandeis Univ. (71-2163)	55,000
Colorado State Univ. (71-2192)	55,000
Colorado, Univ. of (71-2186)	37,500
George Washington Univ. (71-2206)	44,950
Johns Hopkins University (72-3224)	37,500
Kansas, Univ. of (71-2184)	135,000
Little, A.D. (65-61)	175,000
Med. College of Va. (69-2266)	12,346
Missouri, Univ. of (71-2323)	40,000
Oregon State Univ. (71-2191)	21,359
Oregon State Univ. (72-3201)	50,048
Southern Research Inst. (65-654)	174,425
Southern Research Inst. (70-2083)	72,500

TABLE II

(continued)

ACTIVITY CONTRACT	DOLLAR LEVEL[1/]
Stage VI - Toxicologic and Pharmacologic Studies in Large Animals and Man (continued)	
Southern Research Inst. (70-2098)	25,000
Stanford Research Inst. (72-3200)	85,480
Stanford Research Inst. (71-2162)	60,000
Texas, Univ of (67-1143)	50,000
Texas, Univ of (66-1156)	132,000
TRW, Inc. (66-84)	323,483
TRW, Inc. (71-2190)	45,000
Washington, Univ. of (71-2193)	29,278
Stage VII - Clinical Trials - Adjuvant Studies. Determination of Role of Agents in Total Care of Patients	$1,037,200
Armed Forces Inst. (72-64)	19,700
Atomic Energy Comm. (71-61)	17,000
Georgetown Univ. (71-2120)	131,300
Hebrew Univ. (71-2127)	65,000
Makerere Univ. (67-1343)	96,250
Veterans Admin. (64-4)	394,000
Stage -- - Unassigned. Includes Administration, Dissemination of Information to Medical and Scientific Community	$68,217
FASEB (72-428)	15,000
George Wash. Univ. (69-2116)	2,627
3 I Company (71-2306)	3,090
Thompson, J.I., Co. (69-2158)	47,500

1/ Funds obligated during Fiscal Year 1972, not included are funds utilized for direct purchase of clinical drugs (approximately $1,403,483) and about twenty-seven new contracts not negotiated as of this report. ($2,519,000)

TABLE III

ANALYSIS OF ACTIVITIES

BY CONTRACT

CONTRACTOR / FUNCTIONS		DOLLAR LEVEL
Abcor, Inc. (70-2239)		$ 4,000
6X1 Clinical Trials - Phase III		
Aerojet-General Corporation (68-1300)		223,000
2B4 Procur. of agents for pre-clinical studies	100,000	
3X2 Product. & formulation for clinical trials	123,000	
Agriculture, Department of (Transfer 64-1)		337,400
1XX Acquisition of new agents	307,400	
2B4 Procure. of agents for pre-clinical studies	16,000	
3X2 Product. & formulation for clinical trials	14,000	
Albany Medical College (72-2074)		49,736
5XX Clinical Trials - Phase II		
American Red Cross (70-2005)		110,284
6X1 Clinical Trials - Phase III		
Antec Corporation (72-467)		13,575
3X1 Toxicology and pharm. in large animals		
Arizona, University of (67-1484)		104,300
1XX Acquisition of new agents	80,000	
2B4 Procure. of agents for pre-clinical studies	24,300	
Arizona State University (71-2308)		45,000
1XX Acquisition of new agents		
Armed Forces Institute of Pathology (72-64)		19,700
7XX Clinical Trials - adjuvant studies		
ARS/Sprague-Dawley (72-359)		177,840
1XX Acquisition of new agents	24,898	
2AX Determination of anti-tumor activity	112,039	
2B2 Detailed evaluation of new agents	40,903	
ARS/Sprague-Dawley (70-2044)		120,000
1XX Acquisition of new agents	16,800	
2AX Determination of anti-tumor activity	75,600	
2B2 Detailed evaluation of new drugs	27,600	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
ARS/Sprague-Dawley (71-2007)			$ 611,237
1XX	Acquisition of new agents	85,573	
2AX	Determination of anti-tumor activity	385,079	
2B2	Detailed evaluation of new agents	140,585	
ARS/Sprague-Dawley (71-2342)			90,000
1XX	Acquisition of new agents	12,600	
2AX	Determination of anti-tumor activity	56,700	
2B2	Detailed evaluation of new agents	20,700	
Associated Biomedic Systems Inc. (70-2271)			94,750
1XX	Acquisition of new agents	18,950	
6X2	Tox. & pharm. studies in large anim.& man	75,800	
Ash Stevens (71-2314)			68,000
1XX	Acquisition of new agents		
Atomic Energy Commission (71-61)			17,000
7XX	Clinical Trials - adjuvant studies		
Atomic Energy Commission (71-60)			60,000
6X2	Tox.& pharm. studies in large anim.& man		
Atomic Energy Commission (71-56)			19,000
2AX	Determination of anti-tumor activity	10,000	
2B2	Detailed evaluation of new agents	9,000	
Atomic Energy Commission (71-59)			36,400
4X1	Clinical Trials - Phase I		
Battelle Memorial Institute (71-2262)			56,255
3X1	Toxicology and pharm. in large animals		
Battelle Memorial Institute (70-2008)			71,485
1XX	Acquisition of new agents	10,008	
2AX	Determination of anti-tumor activity	45,036	
2B2	Detailed evaluation of new agents	16,441	
Battelle Memorial Institute (71-2182)			460,000
1XX	Acquisition of new agents	69,000	
2AX	Determination of anti-tumor activity	280,600	
2B2	Detailed evaluation of new agents	110,400	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Battelle Memorial Institute (69-2251)			$ 175,000
1XX	Acquisition of new agents	24,500	
2AX	Determination of anti-tumor activity	110,250	
2B2	Detailed evaluation of new agents	40,250	
Bellaire Acres (72-376)			210,600
1XX	Acquisition of new agents	29,484	
2AX	Determination of anti-tumor activity	132,678	
2B2	Detailed evaluation of new agents	48,438	
Bellaire Acres (71-652)			23,310
1XX	Acquisition of new agents	3,265	
2AX	Determination of anti-tumor activity	14,685	
2B2	Detailed evaluation of new agents	5,360	
Bellaire acres (72-729)			56,160
1XX	Acquisition of new agents	7,862	
2AX	Determination of anti-tumor activity	35,381	
2B2	Detailed evaluation of new agents	12,917	
Ben Venue Laboratories, Inc. (66-909)			722,557
2B1	Experimental formulation, development	72,200	
3X2	Product. & formulation for clinical trials	650,357	
Biolabs, Incorporated (72-2007)			9,091
6X1	Clinical Trials - Phase III		
Bionetics Research Laboratory (72-2062)			87,216
6X2	Tox. & pharm. studies in large anim. & man		
Bionetics Research Laboratory (71-2340)			785,900
1XX	Acquisition of new agents	196,500	
6X2	Tox. & pharm. studies in large anim. & man	589,400	
Bowman Gray School of Medicine (68-950)			18,262
6X1	Clinical Trials - Phase III		
Brandeis University (71-2163)			55,000
6X2	Tox. & pharm. studies in large anim. & man		

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Bristol-Meyers Company (69-35)			$ 717,000
1XX	Acquisition of new agents	605,000	
2AX	Determination of anti-tumor activity	80,000	
2B2	Detailed evaluation of new agents	10,000	
3X2	Product. & formulation for clinical trials	22,000	
California, University of (70-2014)			36,968
6X1	Clinical Trials - Phase III		
California, University of (70-2092)			117,000
6X1	Clinical Trials - Phase III		
Charles River Breeding Laboratories (72-730)			55,640
1XX	Acquisition of new agents	7,790	
2AX	Determination of anti-tumor activity	35,053	
2B2	Detailed evaluation of new agents	12,797	
Charles River Breeding Laboratories (72-377)			220,350
1XX	Acquisition of new agents	30,849	
2AX	Determination of anti-tumor activity	138,820	
2B2	Detailed evaluation of new agents	50,681	
Charles River Breeding Laboratories (70-2043)			98,500
1XX	Acquisition of new agents	13,790	
2AX	Determination of anti-tumor activity	62,055	
2B2	Detailed evaluation of new agents	22,655	
Charles River Breeding Laboratories (72-2004)			692,575
1XX	Acquisition of new agents	86,961	
2AX	Determination of anti-tumor activity	436,322	
2B2	Detailed evaluation of new agents	159,286	
Charles River Breeding Laboratories (71-668)			46,875
2AX	Determination of anti-tumor activity		
Charles River Breeding Laboratories (71-2343)			90,000
1XX	Acquisition of new agents	12,600	
2AX	Determination of anti-tumor activity	56,700	
2B2	Detailed evaluation of new agents	20,700	
Central Drug Research Institute (P.L.480 Funds)			(*56,754)
1XX	Acquisition of new agents	28,754	
2AX	Determination of anti-tumor activity	28,000	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Chemical Abstract Service (65-1021)			$ 104,499
	1XX Acquisition of new agents		
Collaborative Research Inc. (66-506)			82,175
	1XX Acquisition of new agents	19,720	
	2B4 Procure. of agents for pre-clinical studies	62,455	
Collaborative Research Inc. (71-2326)			50,000
	1XX Acquisition of new agents		
Colorado State University (71-2192)			55,000
	6X2 Tox. & pharm. studies in large anim. & man		
Colorado, University of (71-2186)			75,000
	1XX Acquisition of new agents	37,500	
	6X2 Tox. & pharm. studies in large anim. & man	37,500	
Connecticut, University of (71-2181)			33,980
	6X1 Clinical Trials - Phase III		
Control Data Corporation (67-1356)			30,773
	5XX Clinical Trials - Phase II	15,387	
	6X1 Clinical Trials - Phase III	15,386	
Cornell University Medical College (69-60)			39,900
	1XX Acquisition of new agents		
Costa Rica, University of (64-31)			700
	1XX Acquisition of new agents		
Delaware, University of (69-2115)			18,791
	1XX Acquisition of new agents		
Dow Chemical Company (71-2313)			90,176
	1XX Acquisition of new agents		
Dow Chemical Company (71-2165)			150,986
	2B4 Procure. of agents for pre-clinical studies	90,986	
	3X2 Product. & formulation for clinical trials	60,000	
Duke University Medical Center (72-2044)			93,300
	4X1 Clinical Trials - Phase I		

TABLE III (Continued)

CONTRACTOR / FUNCTIONS		DOLLAR LEVEL
Duke University (70-2030)		$ 51,218
6X1 Clinical Trials - Phase III		
FASEB (72-428)		15,000
8XX Administration and information functions		
Flow Research Animals, Incorporated (72-378)		162,240
1XX Acquisition of new agents	22,714	
2AX Determination of anti-tumor activity	102,211	
2B2 Detailed evaluation of new agents	37,315	
Georgetown University (71-2123)		287,470
6X1 Clinical Trials - Phase III		
Georgetown University (71-2120)		131,300
7XX Clinical Trials - adjuvant studies		
George Washington University (69-2116)		2,627
8XX Administration and information functions		
George Washington University (71-2206)		44,950
6X2 Tox. & pharm. studies in large anim. & man		
Georgia Institute of Technology (68-1288)		15,000
1XX Acquisition of new agents		
Georgia, University of (71-2311)		34,900
1XX Acquisition of new agents		
Germfree Life Research Center (72-3204)		163,649
1XX Acquisition of new agents	22,911	
2AX Determination of anti-tumor activity	103,099	
2B2 Detailed evaluation of new agents	37,639	
Harlan Industries (72-419)		173,160
1XX Acquisition of new agents	24,242	
2AX Determination of anti-tumor activity	109,091	
2B2 Detailed evaluation of new agents	39,827	
Harlan Industries (71-646)		46,875
2AX Determination of anti-tumor activity		

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Harlan Industries (72-724)			$ 58,240
1XX	Acquisition of new agents	8,154	
2AX	Determination of anti-tumor activity	36,691	
2B2	Detailed evaluation of new agents	13,395	
Harlan Industries (72-3218)			$ 118,779
1XX	Acquisition of new agents	16,629	
2AX	Determination of anti-tumor activity	74,831	
2B2	Detailed evaluation of new agents	27,319	
Hawaii, University of (67-748)			91,000
1XX	Acquisition of new agents	46,000	
2B4	Procure. of agents for pre-clinical studies	45,000	
Hazelton Research Animals (72-410)			11,775
3X1	Toxicology and pharm. in large animals		
Hebrew University (71-2127)			65,000
7XX	Clinical Trials - adjuvant studies		
Horton Laboratories (72-361)			60,320
1XX	Acquisition of new agents	8,445	
2AX	Determination of anti-tumor activity	38,002	
2B2	Detailed evaluation of new agents	13,873	
Horton Laboratories (71-709)			46,875
2AX	Determination of anti-tumor activity		
3 I Company (71-2306)			3,090
8XX	Administration and information functions		
IIT Research Institute (68-1021)			1,439,000
1XX	Acquisition of new agents	100,000	
2AX	Determination of anti-tumor activity	1,233,000	
2B2	Detailed evaluation of new agents	106,000	
IIT Research Institute (67-1141)			149,294
3X1	Toxicology and pharm. in large animals		
Illinois, University of (72-2078)			86,000
1XX	Acquisition of new agents		

TABLE III (Continued)

CONTRACTOR / FUNCTIONS		DOLLAR LEVEL
Illinois, University of (72-3208)		$ 55,375
1XX Acquisition of new agents		
Indian Cancer Research Center (PL 480 Funds)		(*18,966)
1XX Acquisition of new agents	9,966	
2AX Determination of anti-tumor activity	9,000	
Indiana University Foundation (70-2028)		18,000
6X1 Clinical Trials - Phase III		
Institute of Jules Bordet (72-2053)		7,200
1XX Acquisition of new agents		
International Research & Development Corp. (71-2264)		47,658
3X1 Toxicology and pharm. in large animals		
Jackson Laboratories (71-1004)		294,279
1XX Acquisition of new agents	41,199	
2AX Determination of anti-tumor activity	185,396	
2B2 Detailed evaluation of new agents	67,684	
Japanese Foundation for Cancer Research (72-2054)		20,174
1XX Acquisition of new agents		
Jefferson Medical College (70-2136)		50,000
4X2 Initial clinical pharm. studies in man		
Johns Hopkins University (72-3224)		75,000
1XX Acquisition of new agents	37,500	
6X2 Tox.& pharm. studies in large anim. & man	37,500	
Kansas, University of (71-2184)		135,000
6X2 Tox.& pharm. studies in large anim. & man		
Kansas, University of (71-2010)		77,671
1XX Acquisition of new agents	10,874	
2AX Determination of anti-tumor activity	48,933	
2B2 Detailed evaluation of new agents	17,864	

*FY71 Funds

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Kentucky, University of (67-1173)			$ 32,517
6X1	Clinical Trials - Phase III		
Laboratory Supply Company (72-360)			177,840
1XX	Acquisition of new agents	24,897	
2AX	Determination of anti-tumor activity	112,040	
2B2	Detailed evaluation of new agents	40,903	
Laboratory Supply Company (70-2041)			118,191
1XX	Acquisition of new agents	16,547	
2AX	Determination of anti-tumor activity	74,460	
2B2	Detailed evaluation of new agents	27,184	
Laboratory Supply Company (72-733)			59,280
1XX	Acquisition of new agents	8,300	
2AX	Determination of anti-tumor activity	37,346	
2B2	Detailed evaluation of new agents	13,634	
Little, Arthur D. Inc. (65-61)			2,425,000
1XX	Acquisition of new agents	50,000	
2AX	Determination of anti-tumor activity	800,000	
2B2	Detailed evaluation of new agents	400,000	
3X1	Toxicology & pharm. in large animals	300,000	
4X2	Initial clinical pharm. studies in man	700,000	
6X2	Tox. & pharm. studies in large anim.& man	175,000	
Little, Arthur D. Inc. (72-3257)			119,200
1XX	Acquisition of new agents	16,000	
2AX	Determination of anti-tumor activity	79,500	
2B2	Detailed evaluation of new agents	23,700	
Makerere University (67-1343)			250,000
5XX	Clinical Trials - Phase II	57,500	
6X1	Clinical Trials - Phase III	96,250	
7XX	Clinical Trials - adjuvant studies	96,250	
Marshall Research Animals (72-364)			15,225
3X1	Toxicology & pharm. in large animals		
Mason Research Institute (70-2055)			423,118
3X1	Toxicology & pharm. in large animals		

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Mason Research Institute (71-2259)			$ 560,000
1XX	Acquisition of new agents	5,600	
2AX	Determination of anti-tumor activity	489,560	
2B2	Detailed evaluation of new agents	64,840	
Mayo Foundation (71-2185)			98,800
5XX	Clinical Trials - Phase II		
Mayo Foundation (70-2066)			100,000
5XX	Clinical Trials - Phase II		
Medical College of Virginia (69-2266)			12,346
6X2	Tox. & pharm. studies in large anim. & man		
Memorial Hospital (72-2073)			192,627
5XX	Clinical Trials - Phase II		
Merck and Company (72-2002)			507,000
2B4	Procure. of agents for pre-clinical studies	453,000	
3X2	Product. and formulation for clinical trials	54,000	
Miami, University of (70-2225)			80,132
1XX	Acquisition of new agents	32,132	
2AX	Determination of anti-tumor activity	48,000	
Michigan, Department of Health (65-7)			42,655
1XX	Acquisition of new agents		
Michigan, Department of Health (68-1284)			70,000
2B1	Experimental formulation, development		
Michigan, University of (69-2004)			47,886
1XX	Acquisition of new agents		
Microbiological Associates, Inc. (68-1283)			2,210,000
1XX	Acquisition of new agents	600,000	
2AX	Determination of anti-tumor activity	650,000	
2B2	Detailed evaluation of new agents	450,000	
2B3	Initial animal pharm. studies	30,000	
3X1	Toxicology and pharm. in large animals	150,000	
4X2	Initial clinical pharm. studies in man	330,000	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Microbiological Associates, Inc. (69-2177)			$ 135,000
6X1	Clinical Trials - Phase III		
Midwest Research Institute (71-2263)			51,813
3X1	Toxicology and pharm. in large animals		
Midwest Research Institute (65-94)			291,308
1XX	Acquisition of new agents		
Midwest Research Institute (69-2113)			159,000
2B1	Experimental formulation, development	100,000	
3X2	Product. and formulation for clinical trials	59,000	
Missouri, University of (71-2323)			40,000
6X2	Tox. & pharm. studies in large anim. & man		
Monsanto Research Corporation (65-1044)			140,000
2B3	Initial animal pharm. studies	65,000	
3X1	Toxicology and pharm. in large animals	50,000	
4X2	Initial clinical pharm. studies in man	25,000	
Monsanto Research Corporation (67-658)			158,000
2B4	Procure. of agents for pre-clinical studies	38,000	
3X2	Product. and formulation for clinical trials	120,000	
National Academy of Sciences (64-44)(Task Order #6)			17,500
1XX	Acquisition of new agents	2,450	
2AX	Determination of anti-tumor activity	11,025	
2B2	Detailed evaluation of new agents	4,025	
National Academy of Sciences(64-44)(Task Order #16)			20,000
1XX	Acquisition of new agents	2,800	
2AX	Determination of anti-tumor activity	12,600	
2B2	Detailed evaluation of new agents	4,600	
National Bureau of Standards (69-49)			161,000
4X1	Clinical Trials - Phase I		
New Jersey College of Medicine and Dentistry (66-52)			9,761
2AX	Determination of anti-tumor activity	7,330	
2B2	Detailed evaluation of new agents	2,440	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
New Jersey College of Medicine and Dentistry (72-3211)			$ 160,000
1XX	Acquisition of new agents	22,400	
2AX	Determination of anti-tumor activity	100,800	
2B2	Detailed evaluation of new agents	36,800	
New York State Department of Health (72-2072)			78,800
5XX	Clinical Trials - Phase II		
New York State Department of Health (70-2035)			74,953
1XX	Acquisition of new agents	10,494	
2AX	Determination of anti-tumor activity	47,220	
2B2	Detailed evaluation of new agents	17,239	
New York University (67-1174)			25,300
6X1	Clinical Trials - Phase III		
Ohio State University (67-1175)			25,060
6X1	Clinical Trials - Phase III		
Oregon State University (71-2191)			21,359
6X2	Tox. & pharm. studies in large anim. & man		
Oregon State University (72-3201)			50,048
6X2	Tox. & pharm. studies in large anim. & man		
Pennsylvania, University of (71-2187)			150,000
1XX	Acquisition of new agents		
Pennsylvania, University of (71-2188)			10,550
2AX	Determination of anti-tumor activity		
Pennwalt Corporation (71-2309)			59,890
1XX	Acquisition of new agents		
Philips Roxane Labs (71-2139)			138,490
2B1	Experimental formulation, development	24,928	
3X2	Product. & formulation for clinical trials	113,562	
Pomona College (69-2112)			37,251
1XX	Acquisition of new agents		

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Rawley Farms (72-380)			$ 206,700
1XX	Acquisition of new agents	28,938	
2AX	Determination of anti-tumor activity	130,221	
2B2	Detailed evaluation of new agents	47,541	
Research Triangle Institute (69-2019)			139,968
1XX	Acquisition of new agents	100,000	
2B4	Procure. of agents for pre-clinical studies	39,968	
Rhode Island, University of (71-2312)			39,778
1XX	Acquisition of new agents		
Scripps Clinic and Research Foundation (72-2042)			39,000
1XX	Acquisition of new agents	19,000	
2AX	Determination of anti-tumor activity	20,000	
Sheehan Institute for Research (71-2207)			45,000
1XX	Acquisition of new agents		
Simonsen Laboratories (72-3219)			122,367
1XX	Acquisition of new agents	17,131	
2AX	Determination of anti-tumor activity	77,092	
2B2	Detailed evaluation of new agents	28,144	
Simonsen Laboratories (72-409)			176,280
1XX	Acquisition of new agents	24,680	
2AX	Determination of anti-tumor activity	111,056	
2B2	Detailed evalu tion of new agents	40,544	
Simonsen Laboratories (71-657)			12,400
1XX	Acquisition of new agents	1,736	
2AX	Determination of anti-tumor activity	7,812	
2B2	Detailed evaluation of new agents	2,852	
Simonsen Laboratories (71-2101)			179,354
1XX	Acquisition of new agents	25,110	
2AX	Determination of anti-tumor activity	112,993	
2B2	Detailed evaluation of new agents	41,251	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Simonsen Laboratories (72-725)			$ 69,320
1XX	Acquisition of new agents	8,445	
2AX	Determination of anti-tumor activity	38,000	
2B2	Detailed evaluation of new agents	13,875	
Southern Animal Farms (72-379)			165,360
1XX	Acquisition of new agents	23,150	
2AX	Determination of anti-tumor activity	104,177	
2B2	Detailed evaluation of new agents	38,033	
Southern Animal Farms (71-645)			46,875
2AX	Determination of anti-tumor activity		
Southern Animal Farms (72-2013)			121,683
1XX	Acquisition of new agents	17,036	
2AX	Determination of anti-tumor activity	76,660	
2B2	Detailed evaluation of new agents	27,987	
Southern Animal Farms (72-732)			61,360
1XX	Acquisition of new agents	8,590	
2AX	Determination of anti-tumor activity	38,657	
2B2	Detailed evaluation of new agents	14,113	
Southern Research Institute (71-2021)			456,000
1XX	Acquisition of new agents		
Southern Research Institute (65-654)			345,400
4X2	Initial clinical pharm. studies in man	170,975	
6X2	Tox. & pharm. studies in large anim. & man	174,425	
Southern Research Institute (66-29)			502,000
1XX	Acquisition of new agents	250,000	
2B2	Detailed evaluation of new agents	252,000	
Southern Research Institute (71-2189)			27,400
2AX	Determination of anti-tumor activity		
Southern Research Institute (70-2083)			290,000
3X1	Toxicology and pharm. in large animals	217,500	
6X2	Tox. & pharm. studies in large anim. & man	72,500	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Southern Research Institute (71-2098)			$2,043,000
1XX	Acquisition of new agents	100,000	
2AX	Determination of anti-tumor activity	1,225,000	
2B2	Detailed evaluation of new agents	693,000	
6X2	Tox. & pharm. studies in large anim. & man	25,000	
South Shore Analytic and Research Lab (64-932)			179,994
3X1	Toxicology & pharm. in large animals		
Stanford Research Institute (71-2070)			628,000
1XX	Acquisition of new agents	400,000	
2B4	Procure. of agents for pre-clinical studies	150,000	
3X2	Product. and formulation for clinical trials	78,000	
Stanford Research Institute (72-3200)			85,480
6X2	Tox. & pharm. studies in large anim. & man		
Stanford Research Institute (69-37)			27,425
1XX	Acquisition of new agents		
Stanford Research Institute (71-2162)			138,680
2B3	Initial animal pharm. studies	40,000	
4X2	Initial clinical pharm. studies in man	38,680	
6X2	Tox. & pharm. studies in large anim. & man	60,000	
Starks Associates, Incorporated (62-484)			216,000
2B4	Procure. of agents for preclinical studies	108,000	
3X2	Product. and formulation for clinical trials	108,000	
Starks Associates, Incorporated (68-1275)			46,050
2B4	Procure. of agents for pre-clinical studies		
Starks Associates, Incorporated (72-3203)			204,000
2B4	Procure. of agents for pre-clinical studies	162,000	
3X2	Product. & formulation for clinical trials	42,000	
Taconic Farms (72-381)			132,600
1XX	Acquisition of new agents	18,564	
2AX	Determination of anti-tumor activity	83,538	
2B2	Detailed evaluation of new agents	30,498	

TABLE III (Continued)

CONTRACTORS	FUNCTIONS		DOLLAR LEVEL
Texas Inbred Mice (68-88)			$ 432,058
1XX	Acquisition of new agents	60,488	
2AX	Determination of anti-tumor activity	272,196	
2B2	Detailed evaluation of new agents	99,374	
Texas Inbred Mice (72-375)			145,600
1XX	Acquisition of new agents	20,382	
2AX	Determination of anti-tumor activity	91,730	
2B2	Detailed evaluation of new agents	33,488	
Texas, University of (71-2094)			425,000
6X1	Clinical Trials - Phase III		
Texas, University of (67-1143)			50,000
6X2	Tox. & pharm. studies in large anim. & man		
Texas, University of (66-1156)			264,000
4X2	Initial clinical phar. studies in man	132,000	
6X2	Tox. & pharm. studies in large anim. & man	132,000	
TRW, Incorporated (66-84)			323,483
6X2	Tox. & pharm. studies in large anim. & man		
TRW, Incorporated (69-2079)			33,320
3X1	Toxicology & pharm. in large animals		
TRW, Incorporated (63-622)			969,000
1XX	Acquisition of new agents	219,000	
2AX	Determination of anti-tumor activity	500,000	
2B2	Detailed evaluation of new agents	250,000	
TRW, Incorporated (68-1286)			298,450
4X1	Clinical trials - Phase I		
TRW, Incorporated (71-2190)			45,000
6X2	Tox. & pharm. studies in large anim. & man		
Tennessee, University of (70-2084)			80,000
2B1	Experimental formulation, development	20,000	
3X2	Product. and form. for clinical trials	60,000	

TABLE III (Continued)

CONTRACTORS	FUNCTIONS		DOLLAR LEVEL
Thompson, John I, Company (69-2158)			$ 47,500
8XX	Administration and information functions		
Tulane University (65-593)			122,290
1XX	Acquisition of new agents	17,120	
2AX	Determination of anti-tumor activity	77,042	
2B2	Detailed evaluation of new agents	28,128	
Upjohn Company (68-1023)			640,000
1XX	Acquisition of new agents	207,000	
2AX	Determination of anti-tumor activity	190,000	
3X2	Product. and form. for clinical trials	11,000	
4X2	Initial clinical pharm. studies in man	232,000	
Utah, University of (65-1041)			120,000
1XX	Acquisition of new agents		
Veterans Administration (Transfer 64-4)			858,000
6X1	Clinical Trials - Phase III	464,000	
7XX	Clinical Trials - adjuvant studies	394,000	
Virginia, University of (71-2099)			94,920
1XX	Acquisition of new agents	60,920	
2B4	Procure. of agents for pre-clinical studies	34,000	
Vitro Laboratories (69-58)			602,608
1XX	Acquisition of new agents	96,417	
2AX	Determination of anti-tumor activity	403,747	
2B2	Detailed evaluation of new agents	102,444	
Vitro Laboratories (72-2021)			49,470
5XX	Clinical Trials - Phase II	24,735	
6X1	Clinical Trials - Phase III	24,735	
Walter Reed Army Medical Center (64-5)			49,349
5XX	Clinical Trials - Phase II		
WARF Institute (66-494)			694,000
1XX	Acquisition of new agents	392,000	
2AX	Determination of anti-tumor activity	231,000	
2B2	Detailed evaluation of new agents	71,000	

TABLE III (Continued)

CONTRACTOR / FUNCTIONS		DOLLAR LEVEL
WARF Institute (67-747)		$ 108,322
1XX Acquisition of new agents		
Washington, University of (71-2193)		29,278
6X2 Tox. & pharm. studies in large anim. & man		
Washington State University (72-2059)		132,739
1XX Acquisition of new agents	18,583	
2AX Determination of anti-tumor activity	83,626	
2B2 Detailed evaluation of new agents	30,530	
Weizmann Institute of Science (PL480 Funds)		(*48,589)
1XX Acquisition of new agents		
Wisconsin, University of (67-1126)		15,995
1XX Acquisition of new agents		
Wisconsin, University of (71-2275)		83,615
1XX Acquisition of new agents		
Yale University (70-2089)		153,758
3X1 Toxicology and pharm. in large animals		
Yale University (67-1193)		15,000
2AX Determination of anti-tumor activity	10,000	
4X2 Initial clinical pharm. studies in man	5,000	
Yale University (71-2339)		55,000
2AX Determination of anti-tumor activity		

* Not included in dollar totals since no Fiscal Year 1972 funds were obligated; included here for reference since it is an on-going contract.

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE CHEMOTHERAPY PROGRAM

ABCOR, INC. (NIH-70-2239)

The object of this effort is to improve the efficiency of granulocyte procurement using the NCI-IBM Cell Separator.

AEROJET-GENERAL CORPORATION (PH43-68-1300)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS)

Under the transfer of funds agreement with the U.S.D.A. the New Crops Research Division collects approximately 5,000 new plant materials per year for screening. Plant samples are obtained from all parts of the world.

The plants collected by the U.S.D.A. are shipped to the extraction contractor, WARF Institute, Inc. As confirmed active plants appear, the U.S.D.A. recollects in large quantity, on a priority based on the needs of the Chemotherapy Program, at the rate of about 220 recollections per year for shipment to the fractionating chemists. Very large recollections are made, as requested by DR&D, for the preparation of clinical quantities of drugs.

ALBANY MEDICAL COLLEGE (NIH-72-2074)

This new contract is designed to evaluate the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival. It is anticipated that at least 40 patient studies will be done annually.

AMERICAN NATIONAL RED CROSS (NIH-70-2005)

The Red Cross supplies the Medicine Branch and other areas of the National Cancer Institute with 120-150 units of platelet concentrates (fresh) per week for experimental use in the clinical wards.

ANTEC CORPORATION (NIH-72-467)

This contract provides 150 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

ARIZONA STATE UNIVERSITY (NIH-71-2308)

The major objective of this contract is the preparation, from animal, marine and plant specimens, of extracts suitable for bioassay in the NCI antitumor screen. The raw natural product specimens are obtained in most instances by the contractor with emphasis on animal and marine sources. However, on occasion, the specimens for extraction are supplied from other sources. The extracts are prepared in accordance with procedures acceptable to NCI and in amounts suitable for routine screening. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation.

ARIZONA, UNIVERSITY OF (PH43-67-1484)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Recent promising results have been obtained from the extraction of plants collected in Greece, Hawaii and Arizona. Further fractionation is being carried out to isolate the active components.

ARMED FORCES INSTITUTE OF PATHOLOGY (NIH-72-64)

This is a contract for the preparation of anti-human rabbit antilymphocyte serum. It is hoped from this contract to have a preparation which could be standardized and used in various transplantation centers throughout the United States for organ transplantation.

ARS/SPRAGUE-DAWLEY (NIH-70-2044)

This rodent breeding center contract supports a production effort designed to furnish approximately 80,000-90,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

ARS/SPRAGUE-DAWLEY (NIH-71-650)

This procurement contract provides 156,000 BDF_1 (C57BL/6♀ x DBA/2♂)hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated March 1972.

ARS/SPRAGUE-DAWLEY (NIH-71-2007)

This genetic-production center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct segments identified as follows:

Segment A provides inbred, outbred and hybrid rodents in modified conventional environments.

ARS/SPRAGUE-DAWLEY (NIH-71-2007) (Continued)

Segment B provides Syrian golden hamsters with defined but non-pathogenic organisms. A small percentage of the effort is devoted to the development of germfree hamsters and gerbils.

Segment C provides inbred and outbred laboratory strains of rats as germfree and/or with defined microflora.

Segment D provides various germfree inbred, outbred and hybrids of inbred rodents, and the production of counterparts of the various species with defined microflora.

ARS/SPRAGUE-DAWLEY (NIH-71-2342)

This contract provides for the maintenance of a Rodent-Production Center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large scale production colonies.

ARS/SPRAGUE-DAWLEY (NIH-72-359)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

ASH STEVENS, INC. (NIH-71-2314)

The objective of this contract is the synthesis of potential antitumor oxygen and/or sulfur heterocyclic compounds. The relatively unexplored areas of 1,2,4-trithiolanes and cyclic disulfides are to be examined as carriers for sulfur and oxygen heterocyclics, metabolically active functions, and anti-metabolites. Advantage will be taken of any leads which develop during the course of the work to extend the syntheses into new potentially active structural areas.

ASSOCIATED BIOMEDIC SYSTEMS, INC. (NIH-70-2271)

This contract supplies blood and blood cells, human tissue culture cells in large supply for cytotoxicity studies and for use by all members of the Laboratory of Tumor Cell Biology in cell biological studies. The contract also provides immunological consulting and will develop antibodies against purified cellular DNA polymerases provided by senior members of the Laboratory for subsequent use in determining cellular localization of polymerases and rapid assay of serum, other biological fluids, and normal cells for polymerases thought to be novel to neoplastic cells. It will also provide screens of sera from various human cancer patients for antibodies against viral specific proteins like the reverse transcriptase.

ATOMIC ENERGY COMMISSION (NIH-71-56)

The principal objective of this research and development project is to study the cell cycle stage specificity of clinical drugs and drugs in development toward clinical trial. Specifically, drugs are evaluated for their ability to inhibit cells in G_1 arrest. The importance of inhibitors of G_1 (and alternative phases of the cell cycle other than the S-phase) stems from the need to develop drugs which may be specifically cytotoxic to non-proliferating but viable and clonogenic tumor cells. The in vitro method used was established by the contractor and involves (a) the reversible arrest of viable cells in G_1 and (b) the release of G_1 arrested cells permitting progression into the S-phase. The system is particularly suited to the objectives of studies because of the unusually long G_1 phase of the Chinese Hamster cells used.

ATOMIC ENERGY COMMISSION (NIH-71-59)

This contract is designed for joint effort of the AEC Cooperative Group to study localization and to determine the usefulness of Gallium-67 in Hodgkin's disease and other lymphomas and lung cancer.

ATOMIC ENERGY COMMISSION (NIH-71-60)

Over the past several years investigators at Oak Ridge have developed high pressure, high resolution liquid chromatographic techniques used with a variety of analytical instruments for determining the individual constituent chemicals present in blood and urine. Because of preliminary publications indicating that specific methylated guanines and other nucleic acid bases are excreted in the urine of patients with acute leukemia, and because tRNA methylase activity is increased in malignant cells, a survey is being conducted to determine whether specific methylated nucleic acid bases are present in increased amounts in the urine and blood obtained from patients with cancer to include a broad survey of types. If present, these compounds might be used as a potential means for determining not only the presence of a malignancy but also the amount of cancer present. Initial observations indicate elevated amounts of specific types of guanines and nucleic acid bases. Further studies are required to define these observations. Other important classes of compounds, including polyamines, are under consideration. As part of the program, in addition to survey of leads and identification of abnormal chromatographic peaks, studies on the improvement of separation techniques and refinement of analytical instrumentation is in progress.

ATOMIC ENERGY COMMISSION (NIH-71-61)

The purpose of this contract is to do an intensive diagnostic procedure in uranium miners with cytology indicative of carcinoma "in situ" to see if a frank lesion can be located. In miners in whom a frank lesion cannot be located, the use of chemotherapy will be entertained.

BATTELLE MEMORIAL INSTITUTE (NIH-69-2251)

The Contractor maintains a genetic-production center for inbred rodents which

BATTELLE MEMORIAL INSTITUTE (NIH-69-2251) (Continued)

provides breeding animals for large-scale laboratory rodent production colonies and limited numbers for testing and research programs. The profile of categories of these animals includes ten strains.

BATTELLE MEMORIAL INSTITUTE (NIH-70-2008)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents, and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples are screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques and the elucidation of salmonella inhibitory substances associated with certain microorganisms.

BATTELLE MEMORIAL INSTITUTE (NIH-71-2182)

Battelle Memorial Institute conducts in vivo testing of new synthetic materials and natural products of both plant and animal origin, as well as the secondary evaluation of active materials. This testing is being conducted at a rate of approximately 25,000 tests per annum. As per the current DR&D protocols, new synthetic materials are tested in the Leukemia L1210 tumor system and in P-388 if supply permits. New natural products are tested in leukemia P388 and those which display activity in this system are then tested in L1210. Dependent upon the needs of the DR&D program, the Project Officer may change the assay systems. A tumor bank is maintained and the tumors stored are available to DR&D should the need arise. Schedule dependency studies are conducted on active agents to determine the optimal dose, schedule, route of administration and host toxicity. At the Project Officer's request, methodology and/or research is scheduled.

BATTELLE MEMORIAL INSTITUTE (NIH-71-2262)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics and temporal relationship to the termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

BELLAIRE ACRES (NIH-71-649)

This procurement contract provided for the supply of 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the DR&D compound evaluation program. Breed-

BELLAIRE ACRES (NIH-71-649) (Continued)

ing animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated March 1972.

BELLAIRE ACRES (NIH-71-652)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract terminated March 1972.

BELLAIRE ACRES (NIH-72-376)

This contract furnishes approximately 390,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

BEN VENUE LABORATORIES, INC. (PH43-66-909)

This contract is designed to provide a pharmaceutical facility for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile parenteral products. Specifically, the contractor performs the following duties:

1. Solubility and stability studies on chemical agents prior to development of a specific dosage form;

2. Development and production of parenteral dosage forms for clinical evaluation;

3. Stability surveillance of all dosage forms prepared.

All projects are initiated by the Project Officer for the contract.

BIOLABS, INC. (NIH-72-2007)

This contract is a feasibility contract designed to determine whether herpes simplex DNA type I could be purified free of host DNA synthesis. This DNA has been supplied and quality assays are ongoing.

BIONETICS RESEARCH LABS., INC. (NIH-71-2340)

For (a) virus supply; (b) screening a large number of compounds of interest for inhibition of various purified DNA polymerases (cellular and viral); (c) determining the biological activities of some DNA polymerase inhibitors in various biological systems on (1) viral oncogenesis, (2) tissue culture cytotoxicity, (3) cytotoxicity on fresh blood leukocytes (normal and leukemic), (4) maturation of leukemic blast cells in the soft agar plating system of

BIONETICS RESEARCH LABS., INC. (NIH-71-2340) (Continued)

Metcalf-Sachs-Paran, (5) for in vivo direct antitumor effect, remission maintenance, and in prophylaxis (e.g., AKR leukemia and mouse mammary tumors; (d) for facilities for collaborating visiting scientists; (e) for analysis of DNA products synthesized by various cellular DNA polymerases; (f) for purification of the factor(s) released by feeder layers of normal cells which induce maturation of human leukemic blast cells in vitro.

BIONETICS RESEARCH LABS., INC. (NIH-72-2062)

This contract provides normal, spontaneously transformed, and viral transformed tissue culture cells for biochemical and biological studies of the cell cycle, e.g., characteristics of so-called "G_0" cells and for studies of cytotoxicity, mechanism of action, and mechanism of cellular resistance to specific chemotherapeutic agents.

BOWMAN GRAY SCHOOL OF MEDICINE (PH43-68-950)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

BRANDEIS UNIVERSITY (NIH-71-2163)

The principal investigator, Dr. Lawrence Levine, has developed specific immunoassays for selected methylated guanines and other nucleic acid bases in biologic fluids. Because of the greater sensitivity, this contract was established in order for Dr. Levine to evolve radioimmunoassays for these same compounds. The assays eventually will be used to determine the presence and levels of these materials in serum and spinal fluid from selected cancer patients. As a different method of approach, this technique will also be evaluated in comparison to liquid and gas chromatographic methods. An assessment should then be available as to the better, more useful, practical, and sensitive approach in an effort to determine ways of finding and selecting specific materials suitable as biologic markers for cancer.

BRISTOL LABORATORIES (NIH-69-35)

This contract covers a comprehensive effort to obtain new antitumor agents from fermentation broths or other natural products. It includes (1) the preparation of broths, or other natural products, for screening against in vivo systems specified by NCI, (2) a rodent tumor laboratory for in vivo screening and also testing of fractionation and other follow-up studies, (3) the isolation work required to obtain purified agents, (4) development and use of in vitro correlative assays for more rapid follow-up of such samples, (5) production of large quantities of agents approved for clinical trials, and (6) the preparation of appropriate dosage forms of such agents.

CALIFORNIA, UNIVERSITY OF (NIH-70-2014)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

CALIFORNIA, UNIVERSITY OF (NIH-70-2092)

This contractor provides research support for the more complex problems in tissue typing (see PH43-69-2177) in addition to furnishing typing plates and specific antisera. He also makes available to investigators in NCI typing data on a very large population of random donors.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - Agreement No. NIH-01-004-1)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by DR&D, and fractionates the active extracts. During the past three years the budget was increased to provide for (1) synthesis of compounds of chemotherapeutic interest, and (2) setting up an antitumor screening laboratory to provide in-house testing capability. Several compounds have been received by DR&D and limited in-house testing has been achieved.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-70-2043)

This rodent breeding center contract supports a production effort designed to furnish approximately 60,000-70,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-443)

This procurement contract supplied 41,000 Fischer 344 rats yearly for rodent tumor tests. Original breeding animals were derived by hysterotomy and isolator techniques in order to reduce disease levels by eliminating those of major importance. This contract terminated December 1971.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-644)

This procurement contract provides for the supply of 78,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for DR&D compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

CHARLES RIVER BREEDING LABORATOTIES, INC. (NIH-71-668)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for DR&D contract studies. The breeding animals, which originate from the Genetic Center at this site, are from the isolator system and are free from parasites and pathologic organisms. This contract terminated March 1972.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-2343)

This contract provides for the maintenance of a Rodent-Production Center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-72-377)

This contract furnishes approximately 390,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-72-2004)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in jacket isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. A colony of agoutis (Dasyprocta agouti) is maintained in order to furnish serum for L-asparaginase extraction.

CHEMICAL ABSTRACTS SERVICE (PH43-65-1021)

The Chemical Abstracts Service (CAS) is operating an automated NCI chemical information system similar to the CAS Registry System. The data bank contains information on about 178,000 unique compounds submitted to DR&D for screening and on other selected compounds of interest. Included is such information as the chemical structure representation, corresponding substructure fragments, and CAS Registry Numbers for NCI compounds. The magnetic tape files are used to determine which newly-offered compounds duplicate those already in the screen, to carry out substructure searches for analogs of compounds of interest, and to prepare indexes of name and molecular formula cross-referenced to NSC number. Full structure searches are made of large offerings from other collections to identify nonduplicates and thereby facilitate the acquisition of new materials for screening. Some consultation in nomenclature and system analysis is also provided.

COLLABORATIVE RESEARCH, INC. (PH43-66-506)

The major objective of this contract is the production of 5-methyl-tetrahydrohomofolic acid (NSC-139490) in large quantities. This derivative of tetrahydrohomofolic acid (NSC-89473) has improved stability properties over the parent compound and is needed in significant quantities for preclinical

COLLABORATIVE RESEARCH, INC. (PH43-66-506) (Continued)

toxicologic and pharmacologic evaluation. Development work on the large-scale preparation of this substance has also been done under this contract. In addition, the synthesis of some additional analogs and derivatives of homofolic acid (NSC-79249) and cytoxan has been undertaken.

COLLABORATIVE RESEARCH, INC. (NIH-71-2326)

The objective of this contract is the design and synthesis of oligo and polynucleotides as potential inhibitors of RNA-dependent DNA polymerase and other DNA polymerases. It is anticipated that the synthetic nucleotides may act as templates and primers for different enzymes and may inhibit viral and leukemic DNA polymerases specifically without interfering with the normal nucleic acid metabolism of the host cells.

COLORADO STATE UNIVERSITY (NIH-71-2192)

The objective of this research-type contract is to investigate the pathogenesis of Daunomycin-induced cardiotoxicity utilizing sophisticated morphologic and biochemical parameters. Initial investigations involve subacute and chronic studies in several species to determine which animal model best reflects the myocardiopathy as reported in man.

COLORADO, UNIVERSITY OF (NIH-71-2186)

This contract concerns the characterization, purification, and biological studies of a specific and natural (i.e., cellular) inhibitor of RNA methylation which is present in normal adult organs but is non-detectable or absent in embryonic and neoplastic tissues. The inhibitor is under hormonal control and appears to be a major regulating factor for methylation of RNA.

CONNECTICUT, UNIVERSITY OF (NIH-71-2181)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

CONTROL DATA CORPORATION (PH43-67-1356)

This is a data processing contract, in which the results of a brain tumor protocol study are being analyzed, utilizing a proprietary software package, System 2000.

CORNELL UNIVERSITY MEDICAL COLLEGE (NIH-69-60)

The purpose of this contract has been to support the clinical use of asparaginase through basic studies of the nature of the biochemical changes which accompany tumor sensitivity and resistance to the enzyme, as well as investigations into the causes and prevention of undesirable side effects.

CORNELL UNIVERSITY MEDICAL COLLEGE (NIH-69-60) (Continued)

These objectives were to be accomplished in part through study of the chemical and physical aspects as well as the mechanism of action and the selection of inhibitors for the enzyme asparagine synthetase. A related area of study was the search for the identity of the protein or proteins whose limitation, as a result of asparagine deprivation, results in cell death. This contract terminated April 1972.

COSTA RICA, UNIVERSITY OF (PH43-64-31)

The contract is concerned with the collection of native Costa Rican plants and preparation of extracts for testing by DR&D.

Aqueous-alcoholic extracts of native plants, many with reputed medicinal properties, are prepared and sent for testing by DR&D. Plants whose extracts possess reproducible activity are recollected in large quantity and shipped to one of our contract fractionators, where chemical isolation studies are undertaken.

During the current year new plants have been collected and extracted at an annual rate of about 150. Several large recollections have been made and shipped to the fractionator. In all, the fractionator has about a dozen large recollections with nearly all under fractionation.

The contract was terminated after six months in the current year, at the contractor's request, and replaced by a purchase contract.

DELAWARE, UNIVERSITY OF (NIH-69-2115)

Principal contract objectives are (1) to further purify an L-glutaminase from Aerobacter aerogenes for therapeutic and toxicologic testing; this amidohydrolase is active at physiological pH and is inert towards L-asparagine; (2) to determine the amino acid sequence of the active site of L-asparaginase from E. coli and selectively to modify that active site without destroying the catalytic integrity of the enzyme.

DOW CHEMICAL COMPANY (NIH-71-2165)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

DOW CHEMICAL COMPANY (NIH-71-2313)

The objective of this contract is the synthesis of new and novel nitrogen heterocyclic compounds as potential antitumor agents. Specifically, the contractor is preparing compounds containing the pyrazolopyrimidine,

DOW CHEMICAL COMPANY (NIH-71-2313) (Continued)

pyrazolotriazine, indanopyrimidine and cyclobutenopyrimidine ring systems. These materials are postulated to be antimetabolites. All materials prepared, including intermediates, are submitted for antitumor testing.

DUKE UNIVERSITY MEDICAL CENTER (NIH-70-2030)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

DUKE UNIVERSITY MEDICAL CENTER (NIH-72-2044)

This project is concerned with improvement in radioautographic technic and/or resolution. The work has concentrated on the subsystem for measuring any improvement produced. This consists of an artificial labeled substance - tritiated polymethacrylate - microtome sectioned and radioautographed. This would allow modulation transfer function measurements for any given film-processing combination, as well as allowing for incorporation of internal standards in future radioautography in general.

FASEB (NIH-72-428)

This is a contract to support an NCI-sponsored symposium entitled "Fundamental Approach to Cancer Chemotherapy" to be presented at the 5th International Congress of Pharmacology, July 23-28, 1972, in San Francisco, California. The symposium will present to the international community of pharmacologists the most current information on the fundamental aspects of cancer chemotherapy. In addition, the contract will give partial support to other symposia and conferences connected with the Congress.

FLOW RESEARCH ANIMALS, INC. (NIH-71-655)

This procurement contract provides for the supply of 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for DR&D compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated March 1972.

FLOW RESEARCH ANIMALS, INC. (NIH-71-656)

This procurement contract was designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. The breeding animals originated from genetic centers. The mice were offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract terminated April 1972.

FLOW RESEARCH ANIMALS, INC. (NIH-72-378)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGETOWN UNIVERSITY (NIH-71-2120)

The Eastern Cooperative Oncology Group membership consists of 23 cooperative institutions, 18 subsidiary hospitals and 180 members. This operations office provides executive and administrative support for activities of the Group. In addition, the office maintains records of patients on protocols; distributes protocols, randomizations and Group correspondence, etc. The Group's office has been responsible for changing the activities of the ECOG along more disease-oriented lines.

GEORGE WASHINGTON UNIVERSITY (NIH-69-2116)

The purpose of this project is to facilitate scanning and hard copy retrieval of cancer chemotherapy literature by incorporating into an automatic microfilm reader-printer system the documents and/or abstracts comprising those cited in Cancer Chemotherapy Abstracts, (CCA), Volume 1, 1960 through Volume 7, 1966, and further, to provide both cumulative agent and author indexes.

GEORGE WASHINGTON UNIVERSITY (NIH-71-2206)

The principal objective of this contract is to investigate the distribution of antineoplastic and other drugs in spontaneous and induced tumors in dogs and other animal species. To accomplish this objective, basic studies are being conducted to evaluate: (a) techniques for determining blood flow to tumors and in various areas of tumors; (b) techniques for evaluating the kinetics of penetration of antineoplastic agents into the intracellular compartment of tumors. During the past year the investigations have primarily dealt with thermal dilution techniques for measuring blood flow and the use of inulin-^{14}C and methotrexate-^{3}H for measuring intracellular permeability.

GEORGIA INSTITUTE OF TECHNOLOGY (PH43-68-1288)

The objective of the contract has been the total synthesis of the alkaloid, Camptothecin (NSC-94600), and the preparation of analogs and derivatives of it. The approach has been to devise a practical synthesis which would be potentially suitable for scale-up from commercially available starting materials. Due to the reduced clinical interest in this compound, this is the terminal year for this contract.

GEORGIA, UNIVERSITY OF (NIH-71-2311)

The goal of this contract is the preparation of novel nitrogen heterocyclic compounds for evaluation as potential antitumor agents. The classes currently being emphasized are ethynyl oxazolidones and heteroaromatic azepines. Because of the structural similarities of these materials to those of compounds with known CNS activity, compounds prepared under this contract will be screened in intracerebral as well as intraperitoneal test systems.

GERMFREE LIFE RESEARCH CENTER (NIH-72-3204)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

HARLAN INDUSTRIES, INC. (NIH-71-646)

This procurement contract was designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. The breeding animals originated from genetic centers. The mice were offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract terminated March 1972.

HARLAN INDUSTRIES, INC. (NIH-72-419)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

HARLAN INDUSTRIES, INC. (NIH-72-3218)

This contract provides for the maintenance of a Rodent-Production Center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies.

HAWAII, UNIVERSITY OF (PH43-67-748)

This contract provided originally for the collection of a minimum of 500 plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are

HAWAII, UNIVERSITY OF (PH43-67-748) (Continued)

reproducibly active in the DR&D tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Since priority was given to recollections, the general collections were reduced to a very small number. During the current year, the contract was increased to provide again for 500 general collections per year.

HAZLETON RESEARCH ANIMALS, INC. (NIH-72-410)

This contract provides 150 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor.

HEBREW UNIVERSITY (NIH-71-2127)

This contract is a research project concerned with the effects of an extract (MER, methanol extracted residue) obtained from tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity.

HERNER INFORMATION SERVICES, INC. (NIH-72-3244)

This contract provides for the preparation, printing and distribution of Cancer Chemotherapy Abstracts (Volumes 13 and 14, for 1972 and 1973). The price of the annual subscriptions pays the cost of photocomposition, printing and mailing by the contractor. This current awareness service, published monthly, supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy.

HORTON LABORATORY ANIMALS, INC. (NIH-71-684)

This procurement contract provides for the supply of 78,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for DR&D compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract will terminate in May 1972.

HORTON LABORATORY ANIMALS, INC. (NIH-71-709)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract will terminate in May 1972.

HORTON LABORATORY ANIMALS, INC. (NIH-72-361)

This contract furnishes approximately 104,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

3I COMPANY (NIH-71-2306)

This contract provides for the preparation of Volume 12 for 1971 of Cancer Chemotherapy Abstracts, a current awareness service which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume consists of six bimonthly issues and an annual cumulative index.

IIT RESEARCH INSTITUTE (PH43-67-1141)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

IIT RESEARCH INSTITUTE (PH43-68-1021)

The primary purpose of this contract is to provide for the performance of in vivo testing of new synthetic materials and natural products, and also for animal health and utilization studies assigned specifically for problems that arise in various screening laboratories and animal supply contractors. Screening is being conducted according to DR&D protocol at a level of approximately 50,000 tests per year. The lymphoid leukemia L1210 in the mouse host is utilized for all synthetics. New natural products are being tested primarily in the P388 leukemia in the mouse host. The Walker 256 carcinosarcoma in the rat host continues to be used for the follow-up of natural products previously found active in this system if they do not have correlative activity in any other system. Studies with the AKR mouse leukemia have been initiated and an increased effort with this system is planned. The assay systems used, however, are determined by the requirements of the Chemotherapy Program, NCI, and may be changed when specifically requested by the Project Officer.

As a second function, this laboratory also maintains a routine Salmonella spp. and Pseudomonas spp. diagnostic laboratory and provides general animal disease diagnostic services as requested. These studies include virology, bacteriology, pathology and histopathology. In addition, it includes baseline studies to define the microbiological flora and virus contact status of various species of interest to the program. This portion of the contract provides the quality controls necessary for the maintenance of the quality of the animals utilized in the DR&D program.

Methodology or research and development is carried out only at the request of the Project Officer and during this year has been devoted to answering specific questions that arose in the in vivo screening and animal health and utilization programs.

ILLINOIS, UNIVERSITY OF (NIH-72-2078)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work will be obtained, for the most part, through the U. S. Department of Agriculture.

ILLINOIS, UNIVERSITY OF (NIH-72-3208)

The principal objectives of this contract are as follows: (1) to provide the Chemotherapy Program of NCI with samples (not previously available) of a number of Ansamycin-type antibiotics for biological and related testing. This group includes the rifamycins, streptovaricins, tolypomycins and geldanamycin, at least some of which are highly potent RNA-dependent DNA polymerase inhibitors and also have antiviral activity. Thus, detailed study of as many members of this group as possible is considered imperative; (2) to isolate several presently unidentified streptovaricin components in quantity sufficient for characterization and biological evaluation; and (3) to study the metabolic fate of ansamycin compounds by isolating and identifying products formed after in vivo administration, from samples collected by investigators specified by NCI.

INDIAN CANCER RESEARCH CENTRE (PL-480 - Agreement No. NIH-01-010-1)

This agreement provides for the collection and identification of Indian plants, extraction and preparation of crude extracts, and for the continued operation, in Bombay, India, of an in vivo screening laboratory for testing materials, both natural products and synthetics, available in India. A mouse breeding colony is maintained for animal production. Testing is conducted according to DR&D protocols with the lymphoid leukemia L1210 and the lymphocytic leukemia P388 test systems being utilized currently.

INDIANA UNIVERSITY FOUNDATION (NIH-70-2028)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

INSTITUT JULES BORDET (NIH-72-2053)

This project involves the establishment and maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. It is designed to foster close collaboration between European and U. S. investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (NIH-71-2264)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics and temporal relationship to the termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

JACKSON LABORATORY (NIH-71-1004)

This service contract provides for the procurement of approximately 112,600 C57BL/6 and 31,400 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 124,800 AKR inbred mice for compound evaluation studies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NIH-72-2054)

This project involves the establishment and maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. It is designed to foster close collaboration between Japanese and U. S. investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy.

JEFFERSON MEDICAL COLLEGE (NIH-70-2136)

The principal objective of this contract is to provide information on the absorption, distribution, and metabolism of antitumor agents undergoing Phase I studies in patients with neoplastic disease. During the past contract year this group has completed a considerable amount of research on three compounds: dibromodulcitol (NSC-104800), streptozotocin (NSC-85998), and 5-azacytidine (NSC-102816).

JOHNS HOPKINS UNIVERSITY (NIH-72-3224)

This contract is concerned with studies on the biological activities of various streptovaricins, including tissue culture cytotoxicity, viral oncogenesis, and in vivo antitumor effects.

KANSAS, UNIVERSITY OF (NIH-71-2010)

This Contractor maintains a genetic-production center. Its most important function is to secure the maintenance of the strains of rodents that are

KANSAS, UNIVERSITY OF (NIH-71-2010) (Continued)

of interest in DR&D program investigations. Its spectrum includes six strains: C57BL/6, DBA/2, AKR, BALB/c, C3H/He, and A/He. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies.

KANSAS, UNIVERSITY OF (NIH-72-3217)

The objective of this research contract is to undertake to resolve difficult formulation problems. This effort involves the development of new and unique clinical delivery systems for poorly manageable drugs. Project assignments and priorities are initiated by the Project Officer for the contract.

KANSAS, UNIVERSITY OF (NIH-71-2184)

The principal objective of this contract is to measure antitumor drugs and/or metabolite levels in plasma with time and urinary and fecal excretion, after a standardized dose and route of administration, in a sufficient number of patients undergoing treatment for malignant disease, to provide adequate statistical documentation of individual variability in pharmacokinetic behavior and response to those drugs. Methotrexate and arabinosyl cytosine are currently under study.

KENTUCKY, UNIVERSITY OF (PH43-67-1173)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

LABORATORY RESEARCH ENTERPRISES, INC. (NIH-71-379)

This procurement contract provided 150 beagles for toxicologic assays. These animals were raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals were supplied by the Contractor. This contract terminated December 1971.

LABORATORY SUPPLY COMPANY (NIH-71-641)

This procurement contract provided for the supply of 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the DR&D screening program. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated March 1972.

LABORATORY SUPPLY COMPANY (NIH-70-2041)

This Contractor maintains a genetic trust stock colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

LABORATORY SUPPLY COMPANY, INC. (NIH-72-360)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

LITTLE, ARTHUR D., INC. (PH43-65-61)

This is a broad-based major research and development program which has been carefully tailored to the overall mission of the NCI Chemotherapy Program. The task objectives range from routine screening activities, in prescribed in vivo and in vitro systems, to highly specialized exploratory research on the physiological disposition of active agents, neuropharmacological studies, including the development of new methodologies, central nervous system solid tumor studies, etc. The total program is divided into ten major categories, each of which is closely coordinated by a specially selected Assistant Project Officer from the appropriate NCI Chemotherapy Program area.

The Screening and Experimental Therapeutics division is basically concerned with the routine screening, in in vivo systems, of synthetic and natural products, as well as companion studies on route and regimen, the effectiveness of combination chemotherapy, and efforts to develop new and improved screening techniques.

Applied Mathematical and Computer Oriented services provide for the analysis of data developed within the contract for the Chemotherapy program, analysis of the B16 melanoma and Lewis lung screening results in order to establish the criteria for antitumor activity and analysis of solvent treated and positive control screening results and the generation of statistical data thereon.

The Cell Kinetics studies include (1) investigations of hematological recovery in the bone marrow of normal drug-treated mice, for the purpose of selecting optimal therapy regimens and for the possible detection of delayed toxicity for the host, (2) measurement of generation times, doubling times and mitotic indices of solid and ascitic tumors via various techniques, (3) bioassay studies with L5178Y lymphocytic leukemia, and (4) related methodological studies.

The Preclinical Toxicology portion is addressed to routine and special pharmacology and toxicology studies in response to problems which arise in the preclinical and clinical phases of the Chemotherapy Program, together with methodological studies directed to the development of related new methodologies.

The Physiologic Disposition and Biochemistry phase of the effort provides for sophisticated exploratory research on the mode of action and physiological disposition of active agents, and includes a cooperative project with selected clinical groups to measure the metabolic fate and physiologic disposistion of drugs being employed in the management of cancer in humans.

The Neuropharmacology studies are aimed at approaching various toxicological and pharmacological problems relating to the treatment of neoplastic diseases of the central nervous system. Improved techniques are being sought for the administration of agents used to control neoplastic disease in the CNS.

LITTLE, ARTHUR D., INC. (PH43-65-61) (Continued)

The Central Nervous System solid tumor studies are concerned with the development and evaluation of transplantable gliomas in the CNS of Beagle dogs, together with collaborative studies with clinical and pharmacology groups of the NCI to examine the growth kinetics and the behavior of transplantable gliomas to selected agents.

The Cell Culture and Methodology work consists of (1) routine cell culture testing of synthetic compounds, antibiotic filtrates and other natural products according to standard protocols, and (2) special studies for the development of improved methods of studying the influence of drugs on cell growth kinetics. The Contractor employs the spin filter continuous culture techniques developed by this group for the study of the mechanisms associated with cell kill, mode of action and cell uptake of selected antitumor agents, etc.

The Electron Microscopy and Radioautography program provides such services in support of the pharmacology, toxicological, microbiological and solid tumor studies.

The Cryobiology and Tumor Distribution activities provide for the maintenance of a frozen tumor and cell bank, distribution of standard tumor lines to screening laboratories and other research institutions, and methodological studies on the frozen-stored tumor lines.

LITTLE, ARTHUR D., INC. (NIH-72-3257)

This project encompasses the review and analysis of the present DR&D Biological Data Processing System. The objectives are to provide recommendations for improving and expanding the present system in order to better evaluate and summarize primary screening data for the selection of compounds for secondary screening, to develop methods for the evaluation and reporting of compounds tested in the secondary screen, and for incorporating the secondary screening data into the present system. A primary goal is to provide suppliers with reports in a form of most use to them. This involves periodic summary reports of all compounds submitted during a year, the suppliers' laboratory numbers, the NSC number assigned, and systematic chemical names along with a brief English language statement indicating whether the compound showed activity in our present screen according to current criteria.

MAKERERE UNIVERSITY (PH43-67-1343)

This contract supports the Lymphoma Treatment Centre at Makarere University, Kampala, Uganda under the immediate supervision of Dr. John Ziegler. A large group of patients with Burkitt's tumor are available in the area. Studies are progressing in Burkitt's tumor patients, evaluating their response to therapy; immune host factors and metabolism of drugs in African patients. Studies in patients with solid tumors including melanoma, Kaposi's sarcoma and hepatocellular carcinoma are in progress with emphasis on experimental chemotherapy and clinical pharmacology.

MARSHALL RESEARCH ANIMALS, INC. (NIH-71-378)

This procurement contract provided 150 beagles for toxicologic assays. These animals were raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals were supplied by the Contractor. This contract terminated November 1971.

MARSHALL RESEARCH ANIMALS, INC. (NIH-72-364)

This contract provides 150 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MASON RESEARCH INSTITUTE (NIH-70-2055)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of 13 potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

MASON RESEARCH INSTITUTE (NIH-71-2259)

This contract provides for the in vivo antitumor screening of new materials and the detailed evaluation in vivo of new and old drugs of clinical interest. Testing is conducted in accordance with DR&D protocols at a level equivalent to the conduct of 25,000 leukemia L1210 tests per year. Emphasis is placed on screening of new agents against L1210 and the testing of selected drugs in additional systems including Lewis Lung carcinoma, B16 melanoma, and P388 leukemia. Studies involving drug combinations, surgery plus chemotherapy, and development of new screening methods are carried out as directed by the Project Officer.

MAYO FOUNDATION (NIH-70-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between The Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the chemotherapy program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at NCI. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (NIH-71-2185)

This contract supports Phase II studies of new antitumor agents in patients with advanced breast cancer.

MEDICAL COLLEGE OF VIRGINIA (NIH-69-2266)

This contract is concerned with testing bacterial endotoxins of E. coli and Salmonella and exotoxins of Streptococcus and Staphylococcus species for possible synergistic toxicity with selected cancer chemotherapeutic agents. Antidotes for the synergistic toxicities are being sought.

MEMORIAL HOSPITAL (NIH-72-2073)

This new contract is designed to conduct Phase II studies of anticancer agents in patients with metastatic adenocarcinoma of the breast. This will be performed as a cooperative venture between Memorial Hospital and the National Cancer Institute. The Contractor will test selected agents either developed by the Chemotherapy Program or of interest to them. The single agent testing will be performed in line with the Chemotherapy Program linear array.

MERCK AND COMPANY (NIH-72-2002)

This contract provides for the resynthesis of compounds in the amounts needed for clinical trial or for necessary preclinical evaluations. About 50% of the budget is devoted to clinical preparations.

This is the largest and oldest of the DR&D preparative laboratories. Because of their extensive and diverse experience, the Contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which cannot be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (NIH-70-2225)

This contract provides for the in vitro cell culture presumptive screening of plant materials, and the follow-up testing of natural products having demonstrated correlative activity in the DR&D in vivo screening program, as a tool for fractionation. Presumptive screening of synthetic materials is also carried out when the quantity of these materials is not sufficient to do primary in vivo screening. The procedures are those prescribed by DR&D protocols which are based on the methods established by Eagle and Foley. Approximately 8,000 tests at three to five dose levels per test are performed yearly.

MICHIGAN DEPARTMENT OF HEALTH (PH43-65-7)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program, serviced by this assay contract, is financed by the Michigan Department of Health.

MICHIGAN, UNIVERSITY OF (PH43-68-1284)

The primary objectives of this contract are two-fold: (1) the development of parenteral clinical dosage forms of potentially active antineoplastic agents, and (2) fundamental research in seeking out new approaches in the formulation of compounds of limited solubility and/or stability. Part 1 of the contract relates to immediate formulation problems and Part 2 involves longer range studies on new concepts of dosage formulation. Project assignments and their priorities are initiated by the Project Officer of the contract. This contract will be terminated during Fiscal Year 1972.

MICHIGAN, UNIVERSITY OF (NIH-69-2004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-1283)

This particular program is the product of the realignment and gradual consolidation, over a number of years, of individual contracts with this Company (both service and research-oriented) which now forms a single broad-based coordinated mechanism for the detailed investigation of problems which arise in the chemotherapy of malignant disease. The total program is closely integrated scientifically with NCI operations, and the activities are carried out under the daily guidance of professional staff of DR&D and Experimental Therapeutics areas of the NCI Chemotherapy Program. Projects selected for study are those which are determined to be highly pertinent to the needs of the preclinical and clinical programs of the NCI. The contract is divided into eight basic divisions, each of which is closely monitored by an Assistant Project Officer.

Chemotherapy Research - Activities in this Division involve exploratory research in biological and biochemical areas of chemotherapy, and include studies on cell kinetics, the search for and evaluation of new screening methods, studies related to remission induction and maintenance, etc.

Drug Evaluation - This Division is concerned with the quantitative evaluation of the therapeutic effectiveness of drugs, including studies on the optimal conditions for administering both old and new drugs, separately and in combination. The program also includes the conduct of primary screening tests at a rate commensurate with the needs of the DR&D screening effort. In addition, this Division evaluates and applies the utility of new methodologies developed collaboratively with the Chemotherapy Research Division.

Immunology - Investigations in this Division involve basic studies on the immunology of viral oncogenesis which may lead to a better understanding of the means for controlling or suppressing the disease through immunology alone or in combination with chemotherapy.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-1283) (Continued)

Toxicology - The toxicology portion of this contract is directed toward: (1) the study of the effect of oncolytic agents on the cardiovascular system, (2) the mechanism of cardiotoxicity, and (3) development of methods for the prevention or modification of the cardiac effects.

Drug Metabolism - This group functions as a source for the study of the physiological distribution and metabolism of antitumor drugs, with special attention to their relationship to therapeutic effectiveness and toxicity.

Organic Chemistry - This portion of the contract is directed to the synthesis of new compounds of potential usefulness, the examination of chemical problems of drug development, and studies on the structure of new agents.

Drug Distribution - This Division provides services to the NCI for the storage and distribution of drugs and chemicals to various research laboratories throughout the country.

Animal Husbandry - The function of this Division is primarily the operation of a genetic center for mouse strains needed in support of the research activities of the contract. Holding facilities and animal caretaking services are a major function of this project.

MICROBIOLOGICAL ASSOCIATES, INC. (NIH-69-2177)

The Contractor performs routine histocompatibility tissue typing of patients in NCI and potential donors of leukocytes, platelets, or bone marrow.

MIDWEST RESEARCH INSTITUTE (PH43-65-94)

Midwest Research Institute is one of three major synthetic contractors for the DR&D. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to DR&D for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around the synthesis of (1) 1,4-bis(alkoxy-substituted phenyl) butane, crown ethers and tropolones as potential t-RNA o-methyl-transferase inhibitors, (2) receptor site-oriented antileukemic compounds based on the N-O-O triangulation hypothesis developed by this contractor and (3) coralyne and other natural products and their analogs. The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MIDWEST RESEARCH INSTITUTE (NIH-69-2113)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for DR&D. The Contractor determines the identity and purity of these materials developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromotographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation.

MIDWEST RESEARCH INSTITUTE (NIH-71-2263)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocol of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics and temporal relationship to the termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

MISSOURI, UNIVERSITY OF (NIH-71-2323)

Because of the great sensitivity and specificity of gas chromatographic techniques, this contract was developed in order to assess the potential for determining the quantitative amount of specific chemicals in the body fluids of cancer patients which might qualify as biologic markers in assessing disease status. Initial efforts have been applied to methylated nucleic acid bases to complement the effort at the Oak Ridge National Laboratory. At present, suitable derivatization techniques have been developed and appropriate clean-up procedure studied. Evaluation of normal urine to establish baseline data and normal range will be carried out. Then aliquot samples of the same urine studied by Oak Ridge will be analyzed. By this means a comparison can be made to the results obtained at Oak Ridge and an evaluation of the best technique for eventual use of these as biologic markers can be determined. Initial studies are also in progress with polyamines. Derivatization and clean-up procedures are feasible by preliminary studies.

MONSANTO RESEARCH CORPORATION (PH43-65-1044)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

MONSANTO RESEARCH CORPORATION (PH43-67-658)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large-scale extraction, isolation, and purification of natural products required for preclinical and clinical studies.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #16)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of bio-medical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #19)

The purpose of the Task Order is to support the activity of the United States National Committee on the International Union against Cancer.

Since its inception in December 1964, this supporting effort has included travel and subsistance and support of conferences and symposia held in various parts of the world.

NATIONAL BUREAU OF STANDARDS (NIH-69-49)

This interagency agreement provides for a collaborative NCI-NINDB-NBS study on automatic identification of cells and counting of radioautographic grains. A computer-controlled scanning optical microscope and a library of subprograms have been developed and will be used to experiment with different strategies for discriminating among cell types.

NATIONAL DRUG COMPANY (NIH-70-2227)

This contract provided a facility for the development and production of oral and parenteral dosage forms with emphasis on injectable products. All formulated lots were evaluated by the contractor for shelf life stability. This contract was terminated in November 1971.

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (PH43-66-52 and NIH-72-3211)

These contracts have as their major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection of antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NIH-70-2035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling DR&D to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic-production centers.

NEW YORK STATE DEPARTMENT OF HEALTH (HEALTH RESEARCH) (NIH-72-2072)

This contract is designed to provide support in the evaluation of the effects of chemotherapy on the growth and size of breast cancer lesions, toxicity and survival using mutually agreeable protocols. It is anticipated that at least 60 patient studies will be done annually. All patients will have clearly definable lesions not previously irradiated and subject to quantitative measurements.

NEW YORK UNIVERSITY MEDICAL CENTER (PH43-67-1174)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

OHIO STATE UNIVERSITY (PH43-67-1175)

The purpose of this contract is to provide data retrieval support for experi-

OHIO STATE UNIVERSITY (PH43-67-1175) (Continued)

mental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

OREGON STATE UNIVERSITY (NIH-71-2191)

This research-type contract is investigating the mechanism of the delayed marrow toxicity of BCNU and related oncolytic nitrosoureas in lower animal species through a systematic search in several species to determine accurately the timing of the leukopenic nadir which follows single graded injections of BCNU. The exact timing of the toxic suppression of erythropoiesis caused by BCNU is being characterized by studying the rate of uptake of ^{59} Iron into hemoglobin of animals given graded doses of the drug.

OREGON STATE UNIVERSITY (NIH-72-3201)

This new contract was initiated in order to facilitate the acquisition of certain pharmacologic disposition information from animals so that such information would be available prior to initiating clinical trials. The major emphasis will be towards studying how microsomal enzyme systems participate in the metabolism of antineoplastic drugs. If the following information is not available for a given agent, the contractor will study the following: (a) plasma levels and urinary, biliary, and fecal excretion rates of drug and/or metabolites; (b) oral vs. parenteral comparison of studies in (a); (c) isolation and identification of major metabolites. This group has been conducting elegant studies on microsomal metabolism of procarbazine and will apply these talents in trying to study the hitherto elusive metabolism of three nitrosoureas: BCNU (NSC-409962), CCNU (NSC-79037) and methyl CCNU (NSC-95441). Their first phase efforts will involve the use of their excellent radiorespirometry setup in attempting to describe the metabolic fate of the chloro-ethyl group in these drugs.

PENNSYLVANIA, UNIVERSITY OF (NIH-71-2187)

This contract will provide the specifications of a detailed system design for an extended Chemotherapy chemical information system to replace the current semiautomated system. The extended system will provide improved capability to: (1) identify which newly received compounds are new to the Chemotherapy Program, including the flagging of analogues and duplicates of compounds of interest; (2) automatically assign samples to screeners and produce computer-generated shipping documents and communications to suppliers, screeners, and in-house staff; (3) respond readily to a significant increase in samples, with rapid turnover from receipt by the Program to shipment for screening; (4) conduct substructure search with simplified inquiry procedures and structure output; (5) maintain on-line inventory control; (6) maintain improved data files, eliminate several manual files, and furnish a variety of retrieval and reporting requirements; (7) link the chemical and biological data files; (8) assess other data and literature files; (9) maintain management and quality control

PENNSYLVANIA, UNIVERSITY OF (NIH-71-2187) (Continued)

of the system; (10) permit the user to be information-oriented, not computer system-oriented; and (11) expand to a fully interactive system with increased user participation.

PENNSYLVANIA, UNIVERSITY OF (NIH-71-2188)

The purpose of this contract is to determine whether there may be useful anti-cancer agents whose prime mechanism of action is selective inhibition of cellular energy production. To accomplish this a study is being made of the correlation between in vivo anticancer activity and the effects on mitochondrial energy coupling reactions and electron shuttle mechanisms of ascites tumor cells in vitro.

PENNWALT CORPORATION (NIH-71-2309)

The objective of this contract is the synthesis of coordination compounds. Few transition metal coordination compounds have been adequately investigated as potential antitumor drugs. This contract will synthesize coordination compounds of platinum, rhodium, niobium and tantalum. It will also prepare cluster compounds of niobium and tantalum for antitumor evaluation. This area of synthetic investigation may be expanded to include additional classes of metal complexes if screening test results warrant.

PHILIPS ROXANE LABORATORIES, INC. (NIH-71-2139)

This service contract provides the Chemotherapy Program with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxanne Laboratories are packaged, labelled and shipped to NCI for subsequent distribution to clinical investigators.

POMONA COLLEGE (NIH-69-2112)

This project has two major objectives. First is the attempt to derive a structure-activity correlation for anticancer drugs. This will be done using currently available data and the statistical methods of linear regression analysis developed by Dr. C. Hansch of Pomona College. The second objective is the accumulation of fundamental data relating to hydrophobic bonding. These data will be used for further refinement of the structure-activity correlation as well as studies of the mechanisms of interaction of "mini-molecules" (drugs) with macromolecules (enzymes).

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS) (Baltimore, Maryland)

This transfer of funds permits service support by the Hospital of NCI-intramural cancer chemotherapy programs at the USPHS Hospital in Baltimore, Maryland. Programs of clinical pharmacology, clinical use of the newer drugs for the treatment of cancer, and developments in improved patient care are on-going projects.

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS) (Baltimore, Maryland) (Continued)

Initial clinical trials of agents developed entirely within the NCI Chemotherapy Program and fundamental related laboratory research represent significant portions of the program.

RAWLEY FARMS (NIH-71-654)

This procurement contract provided 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated March 1972.

RAWLEY FARMS (NIH-71-669)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

RAWLEY FARMS (NIH-72-380)

This contract furnishes approximately 390,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

RESEARCH TRIANGLE INSTITUTE (NIH-69-2019)

The purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compounds. Plant material used for this work is obtained, for the most part, through the U. S. Department of Agriculture. The Contractor has many plants which are, or have been in some stage of fractionation. Several active agents have been isolated and are currently being subjected to additional evaluation.

RHODE ISLAND, UNIVERSITY OF (NIH-71-2312)

This contract provides for the synthesis of nitrogen heterocyclic compounds with potential antitumor properties. The compounds being synthesized are analogs and derivatives of the imidazolopyrazine and isoxazolopyrazine ring systems. Both systems have structural similarity to purines and have the possibility of interfering with normal purine metabolism.

SCIENTIFIC LITERATURE CORPORATION (NIH-70-2267)

This contract provides for the preparation of Cancer Chemotherapy Abstracts (Volume 11, for 1970), a current awareness service, which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume is published in bimonthly issues followed by an annual cumulative index.

SCIENTIFIC LITERATURE CORPORATION (NIH-71-2306)

This contract provides for the preparation of Cancer Chemotherapy Abstracts (Volume 12, for 1971), a current awareness service, which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume is published in bimonthly issues followed by an annual cumulative index.

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH-72-2042)

The objective of this contract is to prepare and test analogs of the coenzyme form of vitamin B_{12}. These are to consist of alterations in both base and/or sugar of the adenosine portion of the molecule. Synthesis will be either enzymatic or chemical. After establishing identity of the agents, they are to be tested first for cell penetration and anti B_{12} activity in vitro. Potentially useful agents are then to be made in larger quantities for in vivo testing for anticancer activity.

SHEEHAN INSTITUTE FOR RESEARCH (NIH-71-2207)

The objective of this contract is the synthesis of new compounds with potential as central nervous system (CNS) antitumor agents. Specifically, compounds related to ergocoronine, which are CNS active and which may pass the blood-brain-barrier, will be synthesized and evaluated in appropriate intracerebral test systems. The compounds prepared will also be evaluated under standard screening protocols.

SIMONSEN LABORATORIES, INC. (NIH-71-2101)

The Contractor maintains a genetic trust stock colony of 6 inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

SIMONSEN LABORATORIES, INC. (NIH-71-639)

This procurement contract provided 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated in March 1972.

SIMONSEN LABORATORIES, INC. (NIH-71-657)

This procurement contract furnished yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. Breeding animals originated from genetic centers. The mice were offered chlorinated water in order to eliminate or control the incidence of pseudomonads. This contract terminated in March 1972.

SIMONSEN LABORATORIES, INC. (NIH-72-409)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid

SIMONSEN LABORATORIES, INC. (NIH-72-409) (Continued)

mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

SIMONSEN LABORATORIES, INC. (NIH-72-3219)

This contract provides for the maintenance of a Rodent-Production Center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTH SHORE LAB., INC. (PH43-64-932)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of 3-4 potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

SOUTHERN ANIMAL FARMS (NIH-71-640)

This procurement contract provided 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originated from genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated in March 1972.

SOUTHERN ANIMAL FARMS (NIH-71-645)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for DR&D contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

SOUTHERN ANIMAL FARMS (NIH-72-379)

This contract furnishes approximately 312,000 BDF_1 (C57BL/6♀ x DBA/2♂)hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

SOUTHERN ANIMAL FARMS (NIH-72-2013)

This contract provides for the maintenance of a Rodent-Production Center. This center produces stocks and strains of rodents for research and testing

SOUTHERN ANIMAL FARMS (NIH-72-2013) (Continued)

laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN RESEARCH INSTITUTE (PH43-65-654)

The primary objective of this contract is to conduct studies on the development of quantitative methods for the estimation of antitumor drugs and their metabolites in biological media, and to apply these methods to in vivo and in vitro experimental systems for determining drug disposition. During the past contract year studies were conducted on the metabolism of cyclophosphamide (Cytoxan), arabinosyl-cytosine (ara-C), vincristine sulfate, Rifampicin, L-asparaginase, tetrahydrouridine, and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, using in vivo and in vitro systems. These studies are part of an integrated effort to provide more extensive and reliable basic quantitative data on the requirements of drug concentration (c) and time (t) to obtain complete tumor cell kill with minimal toxicity to the host.

SOUTHERN RESEARCH INSTITUTE (PH43-66-29)

The major objective of this contract is to develop basic knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim studies are underway on: (a) sites of action and the metabolism of compounds with antitumor activity; (b) the biochemical basis for resistance to important antitumor agents; (c) the comparative biochemistry of tumors and various normal cells; and (d) study of the dynamics of cell growth and the effects of antitumor agents thereon.

SOUTHERN RESEARCH INSTITUTE (NIH-70-2083)

The purpose of this project is to develop toxicological information in primates and dogs which will allow for the safe, effective use of anticancer drugs in leukemia and in solid tumors. In addition, the project attempts to explore the many problems in definition of toxicities resulting from the use of combinations of antitumor drugs. During the past contract year, studies have primarily dealt with the nitrosourea derivatives, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), (NSC 409962), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), (NSC 79037), and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU), (NSC 95441).

SOUTHERN RESEARCH INSTITUTE (NIH-71-2021)

Southern Research Institute is one of the basic synthesis contractors for the DR&D. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines, ring analogs of purines, and nucleosides; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and

SOUTHERN RESEARCH INSTITUTE (NIH-71-2021) (Continued)

analogs of imidazoles; nucleotides; N-nitrosourea analogs; derivatives of vitamin B_6; aryl and heterocyclic triazenes; metabolites of cytoxan; guanazole derivatives; hydroxamic acid derivatives; and analogs of mitomycin.

SOUTHERN RESEARCH INSTITUTE (NIH-71-2098)

Objectives of this comprehensive research and development contract are: (1) development of biological principles leading to discovery of more effective anticancer therapy, (2) development of improved assays for predicting clinical usefulness, and (3) detailed evaluation of new drugs as potential clinical candidates. Objectives are realized through (a) basic studies of factors influencing growth of spontaneous and transplanted animal tumors, (b) studies of the dynamics of tumor cell proliferation and the effects of drugs thereon, and (c) quantitation of drug activity relative to factors which influence it (e.g., treatment route and schedule). Studies of drug combinations and studies of resistance and cross-resistance are conducted to uncover superior therapies leading to cures of animal tumors and in conjunction with studies of mechanisms of drug action. Mammalian cell culture research and development is aimed at defining the influence of drug concentration and duration of effect at the target cell, and the cell cycle stage specificity of active drugs. Antimicrobial testing is aimed at providing information relative to mechanisms of drug action and at identifying suitable organisms for bioassay of effective drug levels in body fluids. Physiological disposition studies formerly conducted under this contract are now conducted under Southern Research Contract 65-654 (Pharmacology) using methodology conducted under this contract. Other changes in this contract within the past year include substantial increases for in vivo and in vitro screening, detailed evaluation of new and old drugs, and for data processing and analysis. The latter is needed to develop methodology for the reporting of testing against spontaneous tumors such as AKR lymphoma and C3H mammary carcinoma. Activities carried out under this contract are closely integrated with projects conducted under Southern Research contracts 71-2021 (Organic Synthesis); 65-654 and 70-2083 (Pharmacology, Physiological Disposition, and Toxicology); 71-2189 (Biochemical Screening); and 66-29 (Biochemical mechanisms).

SOUTHERN RESEARCH INSTITUTE (NIH-71-2189)

The purpose of this contract is to study the effect of potential anticancer agents on cell membrane formation. This is being approached by determining the effects of these drugs on incorporation of labeled precursors into various membrane constituents such as glycoproteins, sphingolipids, phospholipids, cholesterol, etc.

STANFORD RESEARCH INSTITUTE (NIH-69-37)

The objective of this contract is the preparation of folic acid analogs, derivatives and their intermediates. The major emphasis is devoted to the preparation of selected materials determined to be key compounds by the structure-activity study and comprehensive review which was made in this area. The

STANFORD RESEARCH INSTITUTE (NIH-69-37) (Continued)

assignment of the compounds for preparation, whether new synthesis or re-synthesis, is by DR&D staff. The work on this contract was incorporated into Contract NIH-71-2070 with the renewal of NIH-71-2070 in December 1971 and Contract NIH-69-37 was terminated at that time.

STANFORD RESEARCH INSTITUTE (NIH-71-2070)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested by NCI.

The completion of the synthesis of formycin B has opened the path for the synthesis of additional pyrazolo[4,3-d]pyrimidine C-nucleosides. Work is also in progress in the nucleoside area on investigations with both natural and fraudulent bases, and with modifications in the carbohydrate moiety. A considerable program of folic acid antagonists based upon methotrexate is in progress. Exploitation of leads in the indole alkaloid field is being pursued by synthesis of new indole alkaloids. Compounds related to the known indole alkaloids, ellipticine and olivacine, are under investigation. Special attention is being directed to the study of the chemistry of the antibiotic, daunomycin, in order to develop agents of greater specificity at the molecular level. A modest effort is being extended toward preparing simplified synthetic daunomycin analogs that retain only major features of the parent molecule. Also under investigation is the synthesis of a group of compounds designed to intercalate into DNA in the manner proposed for several antitumor compounds and some unusual heterocyclics.

Over 40% of this contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality of the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, and gas), absorption spectroscopy (ultraviolet, visible, and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NIH-71-2162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

STANFORD RESEARCH INSTITUTE (NIH-72-3200)

This new contract was initiated in order to facilitate the acquisition of certain pharmacologic disposition information from animals so that such information would be available prior to initiating clinical trials. The ultimate goal of these studies would be to determine how soluble enzyme systems are involved in the metabolism of antineoplastic agents. If the following information is not available for a given agent, the contractor will study the following: (a) plasma levels and urinary, biliary, and fecal excretion rates of drug and/or metabolites; (b) oral *vs* parenteral comparison of studies in (a); (c) isolation and identification of major metabolites. This institute has an excellent background in conducting such studies with cytosol (non-microsomal) enzyme systems and hence will emphasize research endeavors with antineoplastic agents in chemical classes likely to be handled by these enzymes.

STARKS ASSOCIATES, INC. (PH43-68-1275)

This contract is for the synthesis and/or resynthesis of nucleosides and their intermediates needed to complete structure-activity studies in this area. The major effort is on the resynthesis of designated nucleosides, selected as key compounds for further study by the comprehensive review and structure-activity evaluation of nucleosides previously carried out. The assignment of compounds for preparation, whether new synthesis or resynthesis, originates with DR&D.

STARKS ASSOCIATES, INC. (NIH-72-3203)

This service preparative contract supplies DR&D with compounds required for drug evaluation studies, with 50% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multi-kilograms. Many of the compounds are potential folic acid antagonists and irreversible inhibitors.

TACONIC FARMS, INC. (NIH-71-651)

This procurement contract supplied 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated in March 1972.

TACONIC FARMS, INC. (NIH-72-381)

This contract furnishes approximately 260,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

TENNESSEE, UNIVERSITY OF (NIH-70-2084)

The objectives of this contract mainly include formulation development studies and small batch production of oral solid dosage forms. The contractor also has the capabilities to prepare parenteral dosage forms in pilot batch lots. All work is assigned by the Project Officer for the contract.

TEXAS INBRED MICE COMPANY (PH43-68-88)

This contract supplies a variety of inbred and hybrid rodents for tumor transplantation; for hybrid production (at Texas Inbred and other animal production contractors); and for compound evaluation studies. Animals are supplied from a colony of 25 strains of rodents. The Contractor also serves as a trust stock repository for the inbred strains of rodents.

TEXAS INBRED MICE COMPANY (NIH-71-658)

This procurement contract supplied 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originated in genetic centers and/or C57BL/6 and DBA/2 production colonies. This contract terminated in March 1972.

TEXAS INBRED MICE COMPANY (NIH-72-375)

This contract furnishes approximately 260,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for compound evaluation studies. Breeding animals are furnished from genetic centers and/or Rodent Production colonies.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (PH43-66-1156)

The purpose of this contract is to carry out studies on the pharmacology and physiological distribution of new antitumor agents in man and in experimental animals. Information of potential clinical usefulness is obtained on the absorption, plasma clearance, distribution, plasma protein binding, metabolism, and urinary and biliary excretion of such agents. Analytical methodology is developed where not previously available. Where applicable, the development of comprehensive pharmacokinetic models with potential predictive value is carried out. Studies are closely interrelated with clinical trials of new agents in progress at the same institution. During the past contract year, studies have been performed on arabinosylcytosine, arabinosyl-6-mercaptopurine, cytosine, 1-β-D-arabinofuranosyl-, 5'-palmitate, β-deoxythioguanosine (β-TGdR), and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC). Studies are currently in progress on the pharmacology of cyclocytidine, 1-propanol, 3,3'-iminodi-dimethanesulfonate (ester) (NSC 102627), and 2-6-dimethyl-4-benzyl-4-demethylrifampicin (NSC 139209).

TEXAS, UNIVERSITY OF (PH43-67-1143)

This contract is concerned with the chronic toxicologic evaluation of antineoplastic drugs which are used for extended remission maintenance therapy and in the treatment of psoriasis, collagen diseases and for immunologic suppression. Answers are being sought to the following questions: (1) will the bone marrow continue to demonstrate reversibility of toxicity during a long course of serial insults; (2) what is the tolerance of the major organ systems to prolonged drug therapy and can function impairment be reversed by withdrawal of drug; and (3) what unusual toxicologic phenomenon might be encountered during the long-term administration of a specific agent? This contract is being terminated in Fiscal Year 1973.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NIH-71-2094)

This contract is designed to test the utility of laminar flow isolation and antimicrobial prophylaxis in consolidation therapy of acute leukemia of adults, i.e., whether in addition to reducing the rate of severe infection in such patients, the duration of remission can be prolonged by superintensive chemotherapy.

THOMPSON, JOHN I., COMPANY (NIH-69-2158)

This contract is utilized to maintain and modify the computer system accounting of the cost of drugs to the National Cancer Institute, and to keep a current record of such costs in total and by such variables as drug name, drug lot number, investigator, study group, NIH staff, clinical and non-clinical use of drugs, and other stratification. Data are derived from the records of the receipt of bulk chemicals or pharmaceuticals, and from clinical drug request and shipment forms.

TRW, INC. (PH43-63-622) (now HAZLETON LABORATORIES, INC.)

This contract provides *in vivo* screening of new test materials, both synthetics and natural products, and it provides subsequent testing of active materials.

The current testing level is approximately 50,000 tests per year. Murine L1210 and P-388 Lymphoid Leukemias are the test systems used routinely for all new materials. Testing against the intracerebrally implanted ependymoblastoma is being initiated, and testing against the intramuscularly implanted Walker Carcinosarcoma 256 has been phased out. Treatment schedule studies and research and development work are conducted as required by the Drug Evaluation Branch. Testing is conducted according to DR&D protocols for testing.

TRW, INC. (PH43-66-84) (now HAZLETON LABORATORIES, INC.)

The major objectives of this contract are: (a) to obtain comparative data on the response of non-human primates to known rodent carcinogens and to materials suspected to be carcinogenic in man; (b) to obtain model tumor systems in primates induced by chemicals or viruses so that potential antitumor agents can be evaluated in these systems before undergoing clinical trial; (c) to evaluate the long-term effects of antineoplastic agents currently used for long-term remissions and in treating diffuse collogen disorders; (d) to make available normal and tumor bearing primates as well as those with preneoplastic changes for physiological disposition, biochemical, immunological and diagnostic (biological marker) as well as chemotherapeutic studies. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

TRW, INC. (PH43-68-1286) (now HAZLETON LABORATORIES, INC.)

This contract provides for microbiological services in support of the NCI Chemotherapy clinical investigations at the Baltimore Cancer Research Center,

TRW, INC. (PH43-68-1286) (now HAZLETON LABORATORIES, INC.) (Continued)

and for special testing pertinent to this program. During the past year the services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, NIH, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators.

TRW, INC. (NIH-69-2079) (now HAZLETON LABORATORIES, INC.)

This contract conducts bone marrow transplantation studies on dogs with lymphosarcoma in order to evaluate the immunotherapeutic effectiveness of this approach in large mammals.

TRW, INC. (NIH-71-2190) (now HAZLETON LABORATORIES, INC.)

This contract is addressed to preclinical studies to determine the intrathecal toxicity of selected antineoplastic drugs in Rhesus monkeys in support of proposed clinical studies of new agents using this route of compound administration in the treatment of brain tumors. The current effort provides for the sequential testing of five new candidate agents during this contract year.

TULANE UNIVERSITY (PH43-65-593)

This contract provides for the establishment of a foundation colony of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities.

UPJOHN COMPANY (PH43-68-1023)

This contract has for its primary objective the development of potentially useful antitumor agents, from fungus and actinomycete fermentation beers, including isolation, purification, characterization, production, biochemistry, and pharmacology efforts.

The Upjohn Company has played a leading role in the exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. The present screen, aimed at the detection of new types of antitumor leads, consists of the testing of crude beers in a cell-cycle specificity system which they adapted to routine testing. Those beers showing activity in this system are tested further for activity in the NCI leukemia P-388 screen and the *in vivo* actives receive top priority in follow-up studies.

Major efforts in this contract are devoted to studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program, and phases of toxicological and pharmacological studies within the capability of the contractor and requested by NCI.

UTAH, UNIVERSITY OF (PH43-65-1041)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotics tubercidin, sangivamycin and toyocamycin and other nucleoside antibiotics, (2) derivatives of the C-nucleoside formycin, (3) pyrazolo[4,3-d]pyrimidine and pyrazolo[3,4-d]pyrimidine nucleosides, (4) imidazo[4,5-c]pyridine, imidazo [4,5-b]pyridine and v-triazolo[4,5-c]pyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. This contractor provides these difficult materials in quantities sufficient for in vivo antitumor evaluation by methods of synthesis developed by this group.

VETERANS ADMINISTRATION (D.C.) (TRANSFER OF FUNDS)

Funding is provided for a cooperative effort between the Veterans Administration and the National Cancer Institute for the clinical evaluation of potentially useful anticancer compounds. At the present time the program includes three elements: (1) the Veterans Administration Lung Cancer Cooperative Group, whose objective is to perform Phase II studies of promising new agents in metastatic lung cancer, (2) the VA Surgical Adjuvant Cooperative Group evaluates combined approaches of surgery plus drugs, and (3) the NCI-VA Medical Oncology Service at the Washington VA Hospital. This unit is responsible for experimental antitumor chemotherapy and related problems with emphasis on Phase I and early Phase II studies in bronchogenic carcinoma.

VIRGINIA, UNIVERSITY OF (NIH-71-2099)

This contract provides for the fractionation and isolation of active agents from plants with confirmed antitumor activity which stem from the program for screening of crude extracts and are assigned to contract chemists. During the current report period a number of crystalline materials were isolated and are now undergoing further study at various stages of evaluation. Several compounds are scheduled for clinical trial.

VITRO LABORATORIES (NIH-69-58)

Data processing services are provided by this contract to the Chemotherapy Program and to the Endocrine Evaluation Branch of General Laboratories and Clinics (GL&C). The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, DR&D (including computerized queries of the data files) and in the bioassay and endocrine-related tumor programs of the Endocrine Evaluation Branch (EEB); (2) completing the documentation of all DR&D programs and contractor's procedures; (3) reprogramming the Update and Reporting subsystem of the EEB data processing system to be run at DCRT's computer facility and preparing specifications for the EEB Publications subsystem; and (4) providing programming assistance for the Clinical Master File System. Final copy for publication is also prepared by computer and conventional means.

VITRO LABORATORIES (NIH-72-2021)

This contract provides for abstraction and computerization of past clinical data concerning the effectiveness of chemotherapy and granulocyte support.

WALTER REED GENERAL HOSPITAL (TRANSFER OF FUNDS)

This cooperative agreement and transfer of funds provides for the participation of Walter Reed General Hospital as a contributing member to the studies of the Acute Leukemia Cooperative Group B. Funds for professional and technical personnel, as well as consultant fees and miscellaneous costs, are provided.

WARF INSTITUTE, INC. (PH43-66-494)

This contract provides for the in vivo screening of new materials and the related assay and/or follow-up testing of active materials. Testing is conducted according to DR&D protocols and the current test level is approximately 38,000 tests per annum. During the present reporting period all new compounds have been tested in lymphoid leukemia L1210 in the mouse host, with natural products also being tested in the P388 lymphocytic leukemia in the mouse. Testing in the Walker 256 intramuscular tumor in the rat host has been reduced to less than 2% of the total test level, since it is currently used only for confirmation testing of materials previously selected for development because of activity in Walker 256. Those materials without correlative activity in other systems will no longer be pursued. Schedule dependency studies on compounds of potential interest for clinical trial are conducted as required by program needs. Methodology and/or research and development is devoted to specific problems that arise in the routine screening of agents and is directed by the Project Officer.

WARF INSTITUTE, INC. (PH43-67-747)

This represents a service contract to prepare 50% aqueous-ethanol extracts of plant material at the rate of approximately 2,500 extracts per year. A semi-quantitative test for alkaloids and tannins has been run on plant materials of sufficient interest to be considered for fractionation. The majority of the contractor's plants are obtained through a transfer of funds agreement between the NCI and the U. S. Department of Agriculture. With the dropping of the Walker 256 tumor from our general screen, the tannin test has been dropped. Recently, the contract has been increased to permit the extraction of more plant materials.

WARNER-LAMBERT RESEARCH INSTITUTE (NIH-72-3250)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for Chemotherapy clinical evaluation. The contractor is responsible for completing required analytical and safety tests on each product prepared. All work is assigned by the Project Officer for the contract.

WASHINGTON STATE UNIVERSITY (NIH-72-2059)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

WASHINGTON, UNIVERSITY OF (NIH-71-2193)

The purpose of this research-type contract is to explore the effects of high doses of chemotherapeutic agents in conjunction with autologous and homologous bone marrow infusions. Emphasis is being directed to the hematopoietic toxicity and immunosuppressive manifestations of the agents. The investigation involves determining which antineoplastic agents or combination of agents that have dose limiting bone marrow toxicity in fact do not produce significant immunosuppression. In addition, it is hoped that these studies will reveal any unexpected drug-induced toxicity that may be associated with unusually high doses of selected oncolytic drugs.

WEIZMANN INSTITUTE OF SCIENCE (PL-480 - Agreement No. NIH-06-025-1)

The objectives of this contract are: (1) to seek selectivity in cancer therapy through modification of the physical properties of anticancer agents by various oligomers and polymers, and (2) to produce polymers of amino acids which in themselves may have anticancer activity. The principal emphasis at present is on the interactions and relationships between these polymers and cell membranes.

WISCONSIN, UNIVERSITY OF (PH43-67-1126)

This contract, utilizing cell culture techniques, provides a rapid assay as an aid in the purification of potential antitumor agents from plant materials. The materials tested are the fractionation products prepared under Contract NIH-71-2099 for the purpose of isolating the active agents from materials having established antitumor activity in the DR&D *in vivo* screen. The principal methods used are an agar diffusion system and bioautography of paper chromatograms.

WISCONSIN, UNIVERSITY OF (NIH-71-2275)

This contract supports work for synthesis, purification, and precise chemical analysis of synthetic nucleic acids to be used as well-defined templates in work on DNA polymerases. It also supports research on the mechanisms of various DNA polymerases and research designed to develop more specific templates that may distinguish various polymerases.

YALE UNIVERSITY SCHOOL OF MEDICINE (PH43-67-1193)

The objective of this contract is to provide in vitro screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of dihydrofolate reductase from L1210 leukemia cells, from human chronic myelogenous leukemia and from normal red blood cells.

Some pharmacologic and metabolic work is done on selected agents of particular interest.

YALE UNIVERSITY SCHOOL OF MEDICINE (NIH-70-2089)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of three potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

YALE UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2339)

The objective of this contract is to select possible anticancer agents by determining their effects on the several nucleic acid polymerases. Measurements will be made of alterations in strength of binding of the drugs to various types of double-stranded templates. From this information it is expected that useful anticancer agents which interfere with nucleic acid synthesis and/or transcription can be selected.

SUMMARY

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR PROGRAM

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

The Office of the Associate Scientific Director for Program was created during the last fiscal year partly due to expanded program in Chemotherapy and partly as a consequence of the increased national effort in cancer research. This office has principal responsibility for assisting the Scientific Director for Chemotherapy in effective coordination and integration of the various organizational segments of the Chemotherapy Program with other groups both within the NCI and within the NIH, with efforts funded under contract, with grant supported cooperative groups and with other organizations if their activities are relevant to the plans, objectives and programs of the Chemotherapy Program.

The National Advisory Cancer Council provides guidance for broad policy and program of the National Cancer Institute. Within this framework, the Chemotherapy Advisory Committee assists and advises the Scientific Director for Chemotherapy in programs relevant to the objectives of the Chemotherapy Program as described in the Linear Array.

The Chemotherapy Program has as its objective the control of cancer through the use of chemicals or chemicals in combination with other modalities of therapy. To accomplish this a drug development program has been established at the NCI which serves as a focal point for the national effort in this area. The program is largely implemented through efforts supported by contracts with industry, universities, and non-profit organizations and encompasses every phase of drug research from acquisition of new drugs, identification of anti-tumor activity, fundamental research into new leads to reverse the neoplastic transformation, pharmacokinetics, tumor and normal cell kinetics, toxicology, introduction of new drugs into clinical trials, and the rapid dissemination of new information related to cancer chemotherapy.

These program plans are translated into the logic of the Chemotherapy Linear Array, and as a continuing process, the Linear Array during the past year has been refined consistent with changing plans and priorities. For the first time, the budget for the Chemotherapy Program was allocated according to the stages of the Linear Array and a computer program was developed to permit retrieval of budget information on segments of the Program, both in the collaborative and in the intramural areas.

Program goals and objectives are formulated and implemented by the Scientific Director for Chemotherapy and senior program leaders with a constant effort to predict approaches most likely to result in success in

the shortest possible time. Developments in the scientific and medical world both here and abroad are constantly monitored in the search for potential new leads which might be exploitable in the control of cancer. One of the most important monitoring activities in the Chemotherapy Program concerns surveillance for new anti-tumor agents developed by independent investigators both in this country and abroad. Once identified, these leads are evaluated in depth usually with the help and consultation of a technical review committee and plans are formulated to support an expanded effort in the area specified.

Implementation of these decisions must be considered with relation to resources available at NIH (space, personnel, beds, etc.). Since these resources are limited, the Chemotherapy Program supports a sizeable collaborative effort with universities, non-profit organizations, and industrial firms here in the United States as well as abroad. Senior staff members serve as project officers and are generally selected on the basis of their competence in the area for which the contract is awarded.

Although most collaborative efforts originate in-house, any qualified individual may submit a research proposal for consideration. All proposals however are reviewed for relevance to program priorities, timing, and availability of funds as well as technical quality. The papers are processed according to a tightly structured series of steps designed to insure the incorporation of the foregoing factors in the review mechanism.

Following the establishment of the Office of the Associate Scientific Director for Program in Chemotherapy and similar offices in the other major program segments in the National Cancer Institute, a committee was created to coordinate the activities of the Institute. This Coordinating Committee is chaired by an Assistant Director from the OD, NCI, and its membership is composed of the Associate Scientific Directors for Program.

The Coordinating Committee has had difficulty in accomplishing its charges of (1) coordinating programs in the Institute in order to avoid unnecessary duplication and (2) making recommendations to the Scientific Directorate in problems affecting the Institute as a whole. There have been a number of instances of overlapping programs or duplication which have been identified; but, in general, communication could be greatly improved. The explanation for this lack of success probably lies in at least two factors: (1) decentralization in at least two of the four major divisions of the Institute making it difficult for the ASD for Program to be fully aware of all significant developments in his area and (2) inadvertant omission of the Coordinating Committee from the communication process. Clearly as the Institute expands, its program and activities, particularly in the collaborative and grant supported areas, problems of coordination and communication will become more acute and some mechanism will have to be devised to avoid serious problems.

The Office of the Associate Scientific Director for Program was designated by the Director, NCI, as the focal point for the Institute in the planning and construction of a new ambulatory care addition to the Clinical Center as authorized under the supplemental cancer bill of 1971. There have been

difficulties in reaching agreement on several points including the size of the addition, the allocation of space, need for consolidation of related activities in one geographic location, etc. At this time, a planning firm has been engaged and is in the process of assessing needs with the objective of developing a number of options for consideration by NIH.

When the Office of the Associate Scientific Director for Program, Chemotherapy, was created, the bioengineering effort of the Chemotherapy Program was assigned to the Office. The Program has long had an interest in bridging the gap with industrial engineering competence and biomedical science and has supported a number of successful bioengineering projects including the development of NCI-IBM Cell Separator, the Portable Laminar Air Flow Isolator for patient protection, a device for automated grain counting and image processing (white blood cells), etc.

A bioengineering resource contract is in the process of being awarded to provide engineering competence for selected problems in the Chemotherapy Program. This type of engineering support is a pilot effort and is reviewed as an experimental approach to the support and solution of complex problems which appear to lend themselves to a bioengineering attack. The successful engineering efforts in Chemotherapy are in large part due to the fact that a rather unique situation for NIH exists of having an engineer (there are now two) on the staff of a biomedical area of an institute. In this instance, this has been a very fruitful relationship probably applicable to selected areas elsewhere at NIH.

Serial No. NCI-3409
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971, through June 30, 1972

Project Title: Leukocyte Kinetics and Characteristics of Cell Proliferation

Principal Investigators: Seymour Perry and Mitsuhiro Omine

Other Investigators: Kwang B. Woo, William Z. Penland, Jacqueline Whang-Peng, and Marc Lippman

Man Years:

Total: 2.35
Professional: 1.35
Other: 1.0

Objectives:

1. To investigate the kinetic proliferation and cell cycle characteristics of leukemic blast cells in patients with leukemia and in experimental animals in an attempt thereby to give a firmer theoretical grounding to the devising of more effective therapy.

2. To define the characteristics of the various phases of the cell cycle with emphasis on the resting cell (G_0).

3. To evaluate the effect of anti-tumor agents on normal and tumor cell population kinetics.

Methods Employed:

1. Leukemic or other cells from animals or man are separated, principally on the basis of size, in a special chamber containing a density gradient (sucrose, fetal calf serum, etc.). Separated cells are studied for DNA content, uptake of tritiated thymidine, and proliferative capacity as determined by transplantation and other techniques.

2. Peripheral leukocyte kinetics are studied with the use of $Na_2{}^{51}CrO_4$ and $DF^{32}P$ as labels in vitro. Following infusions, specific radioactivity is monitored.

3. Surface scanning over organs in patients with a dual probe collimated scintillation probe is utilized to study the organ distribution of infused 51-chromate labeled cells.

4. Tritiated thymidine is administered intravenously in man or animals to investigate kinetics of cellular proliferation of both normal and tumor cell populations.

Major Findings

1. Spontaneous AKR leukemia (mice) is considered a good model for human acute leukemia. Thymic lymphoblasts obtained from leukemic animals were separated as described above. The smaller cells were not in DNA synthesis and had a normal complement of DNA. The largest cells had twice the normal complement of DNA and the majority were in DNA synthesis. Between the extremes there were intermediate values of DNA content. However, all cells were capable of inducing leukemia in normal mice, indicating that if the small cells were in G_0, they were readily capable of recycling.

2. Utilization of the chamber separation technique has permitted an analysis of the cell cycle times of spontaneous AKR leukemia. Cell division occurs at approximately 18 hour intervals; T_s is 7 hours; G_1 + 1/2M, 9 hours; and G_2 + 1/2M, 2 hours. Examination of the percent labeled mitoses curve confirms these estimates.

3. Intensive therapy of animals with advanced spontaneous AKR leukemia resulted in the survival of a morphologically uniform population of small cells which are difficult to identify, but they appear to be "resting" leukemic cells.

Proposed Course of Project

1. Studies of the effects of anti-tumor agents in spontaneous AKR will be continued.

2. It is hoped to extend these studies to patients with acute leukemia.

Honors and Awards

None

Publications

1. Perry, S.: Proliferation of myeloid cells. Annual Review of Medicine 22: 171-184, 1971.

2. Rosen, P. J.; Eyre, H. J.; and Perry, S.: Radioisotope labels for the study of leukocyte kinetics. Part I. European Journal of Clinical and Biological Research XVI: 635-641, 1971.

3. Rosen, P. J.; Eyre, H. J.; and Perry, S.: Radioisotope labels for the study of leukocyte kinetics. Part II. European Journal of Clinical and Biological Research XVI: 752-759, 1971.

4. Omine, M., and Perry, S.: Use of cell separation at 1 g for cytokinetic studies in spontaneous AKR leukemia. J. Nat. Cancer Inst. 48: 697-704, 1972.

Serial No. NCI-6401
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971, through June 30, 1972

Project Title: Data Processing Systems for Scientific and Management Information in the Chemotherapy Program

Principal Investigator: Meyer H. Gordon

Other Investigator: Leon Rothenberg

Cooperating Units: Clinical Oncology, Chemotherapy; DCRT

Man Years:

Total: 1.7
Professional: 1.3
Others: 0.4

Objectives

1. Transfer of responsibility for clinical data processing from the Program Analysis Branch to the Clinical Oncology area.

2. Establish data files to be used for the classification of the Chemotherapy Program's research with respect to stage of the linear array logic, subclassification (linear or supplementary array) and National Cancer Program Objective (Approach 6).

3. Develop a system to be used for the storage and retrieval of scientific information by cross-referencing between disease and drug categories.

4. Coordinate data pertaining to computer-oriented expenditures within the Chemotherapy Program.

Methods Employed

1. The major clinical data processing system transferred from the Program Analysis Branch to the Clinical Oncology area was the On/Off Study System. A Clinical Data Reduction contract was initiated with Vitro Laboratories to provide assistance in the preparation of input data for the On/Off Study programs as well as for the maintenance and use of these routines. Minimal effort was expended to maintain the Clinical Masterfile, since there is ongoing development by the Office of Clinical and Management Systems of the Clinical Center and the Division of Computer Research and Technology (DCRT) which will eventually supersede these files.

2. Three WYLBUR files have been created with information pertaining to contracts, Schedule A, and Direct Operations. These files are updated on a monthly basis. All entries in the three files are classified with respect to stage of the linear array logic, subclassification (linear or supplementary array) and National Cancer Program Objective (Approach 6).

3. Work has begun on the development of a system that is disease oriented, cross-indexed to single and combination agents, easily updated, and containing readily retrievable information. A mockup of such a file with appropriately indexed entries has been prepared.

Major Findings

1. On/Off Study material has now been transferred completely to the Clinical Oncology area and is fully operational. The Cell and Platelet Collection systems and the Infusion system are also being transferred from this office to the Clinical Oncology area.

2. Programs have been written to generate reports by various combinations of the three classifications (linear array stage, subclassification, approach category), as well as by Chemotherapy Program organization, from the information contained in the Contracts, Schedule A, and Direct Operations files.

3. With respect to the computerized Drug and Disease-oriented file, discussions with DCRT personnel have indicated that several systems in the process of development may be applicable.

Significance to Biomedical Research

Classification of the Contract, Schedule A, and Direct Operations data by linear array stage, subclassification and National Cancer Plan approach category with the subsequent generation of reports using these categories will lead to a detailed description of the Chemotherapy Program's past, present and future research. The establishment of a Drug and Disease-oriented information system will be of great value in the management of the Chemotherapy Program effort.

Proposed Course

In cooperation with DCRT further development of the Contract, Schedule A, and Direct Operations files will be undertaken, especially for the entry of data and the generation of reports by non-programmers. Similarly, a joint effort with DCRT is anticipated as soon as sufficient data has been gathered to create a comprehensive model of the prototye Drug and Disease-oriented system. This office will become a central clearinghouse for DCRT accounts within the Chemotherapy Program in the future.

Honors and Awards

None

Publications

None

Serial No. NCI-6402
1. Office of the Associate Scientific Director for Program
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971, through June 30, 1972

Project Title: Tumor Growth Kinetics and Chemotherapy

Previous Serial Number: NCI-6109

Principal Investigator: Stanley E. Shackney

Other Investigators: None

Cooperating Units: Dr. Lewis Lipkin, Pathology Section, PRB, NINDS

Man Years:

Total: 2
Professional: 1
Others: 1

Objectives:

1. To develop a model which will describe the growth of tumors in such a way that response to various chemotherapeutic agents can be simulated.

2. To apply the growth model to specific tumor systems.

3. To explore the pharmacodynamics of chemotherapeutic agents in specific tumor systems with the aid of the model.

4. To develop an approach to chemotherapy of tumors based on the expected interaction between tumor growth kinetics and drug pharmacodynamics.

Methods Employed:

Computer modeling techniques, in vivo and in vitro cell propogation techniques, radioautography with tritiated thymidine.

Major Findings:

1. Modelling Studies

A general purpose simulation system has been developed for proliferating mammalian cell populations and their responses to various perturbations.

The properties of growth curves were studied last year. This work has been accepted for publication in the Journal of Theoretical Biology.

Over the past year, the model has been expanded to permit the simulation of tritiated thymidine studies. Simulated PLM curves were explored under a variety of conditions, and compared with the corresponding biological data available from the literature. The following variables were studied in relation to PLM curve characteristics:

a. Population growth rate characteristics in early and late growth,
b. Tritiated thymidine dosage,
c. C x T characteristics of the tritiated thymidine pulse,
d. Emulsion exposure duration, and
e. Grain counting threshold.

The simulations were found to be in agreement with both the experimental and clinical data. Major findings and implications of this work are these:

a. Tritiated thymidine dosage, pulse characteristics, emulsion exposure duration, and grain counting threshold markedly affect the shape of the PLM curve. These factors must be accounted for in any attempt to extract information concerning population growth characteristics for PLM curve data.

b. Because of the low signal detection efficiency of radioautography, the normal occurrence of low levels of DNA synthesis during non-"S phase" portions of the cell cycle cannot be excluded. In fact, currently available evidence indicates that low level DNA synthesis can be demonstrated under conditions where signal detection efficiency is increased, or the signal is amplified.

These conclusions are supported by data collected in our own laboratory (see below).

This work is currently being prepared for publication.

2. Laboratory Studies

a. PLM Curve Studies

We chose to study the effects of two population-independent factors that might affect PLM curve characteristics--namely, emulsion exposure duration, and grain counting threshold.

Studies were initiated in an in vitro system, where tritium pulse C x T characteristics could be better controlled than in vivo. The Foley line of Sarcoma 180 cells was chosen for the following reasons:

(1) It is a hardy cell line, which will grow in a variety of media and under a variety of conditions.

(2) It is a well studied line requiring a minimum number of preliminary characterization studies.

(3) It is transplantable back and forth between mouse and culture flask. In vivo, it will grow as a quantitable solid tumor subq. or i.m., and it will also grow in ascitic form. The solid tumor form has growth characteristics that are desirable for the study of slowly growing tumors and their response to chemotherapy. It is expected that these features will permit comparisons between in vivo and are in vitro kinetic characteristics and drug response characteristics in later stages of the laboratory effort.

Specimens from a three day culture were collected at 2 hour intervals for 24 hours post pulse, and prepared for radioautography. Subsets of slides were exposed for 10, 20, and 40 days. For each set, PLM curves are being collected at 9 grain counting thresholds. To date, the family of PLM curves obtained after 10 days of emulsion exposure has been counted.

From the results obtained so far, it is clear that the shape of the PLM curve is indeed dependent on counting threshold, in agreement with the model's predictions. Lightly labelled mitotic cells which would be scored as unlabelled at high grain counting thresholds are scored as labelled at lower counting thresholds. The heights of the peaks and depth of the trough of the PLM curve are altered accordingly.

b. Bone Marrow Studies

The study of bone marrow kinetics has, in our opinion, been limited by:

(1) Absence of cytoarchitectural landmarks in needle aspirated material.

(2) Uncertainty of the cell classification schema in Romanowsky stained material.

We have, therefore, designed a study in which the cytoarchitecture of bone marrow populations will not be disturbed and in which any biasing of data by a preconceived cell classification scheme will be minimized or avoided.

Fifty mice were injected with a pulse dose of tritiated thymidine and sacrificed by perfusion fixation at intervals ranging from 2 hours to 22 days post pulse. Femurs were decalcified in EDTA, embedded in paraffin, and sectioned at 4 microns. At this writing, the last group of these slides is being processed.

Further processing will include:

(1) Staining with Luxol Fast Blue, periodic acid-schiff, and hemtoxylin, and preparation of radioautographs. These slides should provide a qualitative description of the migration of label with time post pulse through the various morphologic cell forms, in relation to the cytoarchitecture of the bone marrow.

(2) If the qualitative results prove promising, and if a micro-spectrophotometer is available, slides will be stained with Feulgen stain, and DNA content measurements will be performed. Microscopic fields will be marked and photographed, and cells on which DNA content measurements were obtained will be identified on the photographs. Slides will then be prepared for radioautography, and grain counts obtained for those cells whose DNA content was previously measured. This study is far more involved technically but will yield more quantitative data.

c. The Collaborative Effort with the Image Processing Project

The following work was done in response to the needs of the Image Processing Project:

(1) In anticipation of the development of the capability of automated measurements of DNA content and grain counting on the same cells in sectioned material, attempts were made to prepare thin-sectioned DNA standards. Calf thymus DNA was prepared in concentrations ranging from .25 - 1.0 gms/100 cc, and embedded in polyacrylamide gel with the help of Dr. Andrew Peacock. Blocks of this material were fixed in formalin, embedded in Epon, sectioned at 2-5 microns, and stained with Feulgen.

At these section thicknesses only the highest DNA concentrations employed stained intensely enough to be considered. However, the viscosity of the DNA solutions at 1 gm/100 cc was so high that uniform mixing with the polyacrylamide solutions could not be achieved, and bubbles could not be eliminated from the gel.

Alternatively an attempt was made to substitute gelatin for the polyacrylamide. It was found that DNA-gelatin solutions did not gel properly, even after prolonged exposure in the cold, and that they flaked when fixed with formalin.

It was suggested to the image processing group that spermatozoa could serve as a DNA standard. The concentration of DNA in these cells is much higher than any that can be achieved in the laboratory, and DNA content is uniformly haploid.

(2) Documented scans of bone marrow radioautographs were performed in order to provide data for the study of specific problems that were

of interest to the image processing group. These included scans of labelled leukemic blast cells in white and in blue light, with focus on cell and focus on grains, and serial optical sections of labeled blast cells.

(3) Requests for histological material and radioautographs were met when feasible. Processed radioautographs of cultured sarcoma 180 cells, stained either with acetic orcein or Feulgen stain were provided. One hundred and fifty unstained and undipped slides of sarcoma 180 cells labelled with tritiated thymidine were provided.

(4) A question-answer type scan documentation sequence was written in FORTRAN II on the PDP-10 computer.

Significance to Biomedical Research and the Program of the Institute:

Hopefully, a better understanding of the growth characteristics of tumors, and the way in which growth affects response to chemotherapeutic agents, will lead to a rational, systematic approach to the chemotherapy of tumors, both experimentally and clinically, and provide the means for translating experimental data obtained in animals into clinically useful information.

Proposed Course:

1. Modelling Studies

Additional work on tritiated thymidine tracer simulation studies is expected to continue through the summer of 1972. Simulations of long-term infusion studies, DNA content (or cell size) distributions, and combined parameter studies are planned.

The next major modelling effort will be directed toward the modelling of:

a. multiple interdependent populations growing in the same host, and
b. drug effects on growing populations.

The first of these will permit simultaneous comparisons between the simulated responses of normal and tumor cell populations to the same drug schedule. Alternatively, the response of a primary tumor can be compared with that of metastases. Revision of the model to meet these two objectives has already begun.

It is hoped that the major part of the chemotherapy simulation package will be completed by the summer of 1973, and that it will be possible at that time to begin formulating practical chemotherapeutic strategies for clinical cancer chemotherapy.

2. Laboratory studies

It is anticipated that the in vitro PLM curve study will be completed by fall of 1972. Subsequent plans include in vivo studies for comparison with the in vitro work, and a study of the effects of drug administration on mitotic labelling intensity with time post pulse both in vivo and in vitro. The objectives of the drug experiments shall be to study:

a. The differential effects of a given drug on rapidly proliferating and slowly proliferating cells within the same population
b. The differential effects of a given drug on cells engaged in brisk DNA synthesis and cells with low or non-detectable DNA synthesis rates
c. The realtionship of drug dosage to these differential effects
d. The time required for recovery from sublethal damage
e. The susceptibility of sublethally damaged cells to further drug effect.

With regard to the bone marrow study, the first phase should be completed late in fall of 1972. The results of the first phase will determine the course of subsequent work in this area.

3. Collaboration with the Image Processing Project

It is anticipated that the collaborative effort with the image processi project will not be continued.

Honors and Awards

None

Publication

Shackney, S. E.: A cytokinetic model for heterogeneous mammalian cell populations. J. Theoretical Biol. (in press).

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT (DR&D)

CHEMOTHERAPY

Drug Research and Development (formerly Cancer Chemotherapy National Service Center [CCNSC]) has as its primary task the search for and development of new and potentially more effective antitumor agents, as well as the development of better methods of drug selection and prediction of optimal therapy with new and old drugs and drugs combined with other treatment modalities. With respect to the Chemotherapy Program Linear Array, the organization is responsible for all pre-clinical work in the development of drugs except that concerned with toxicology and pharmacology. It also includes information and communication functions in support of other portions of the Chemotherapy Program.

The DR&D program includes both in-house laboratory operations and contracts with pharmaceutical and chemical companies, research institutes and academic institutions, with the major portion of the funding directed toward the contract-supported activities. The program depends heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts. The program activities cover the following specific areas:

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for pre-clinical and clinical evaluation.
3. Development of new agents from fermentation, plant and other natural products.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Primary screening and evaluation of new materials.
7. Bioassays, both *in vivo* and *in vitro*, to monitor the purification of fermentation and other natural products.
8. Study of optimal drug routes and regimens, including combination therapy and other means for improving drug effectiveness.
9. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential antitumor agents.
10. Investigation of mechanisms of action and resistance of drugs of clinical interest.
11. Animal procurement, distribution and disease control.
12. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
13. Development and operation of data processing systems for both biological and chemical information.
14. Search, retrieval and dissemination of pertinent information from the world's literature on drugs and other aspects of cancer therapy.
15. Publication of the abstract journal, *Cancer Chemotherapy Abstracts*.
16. Publication of the scientific journal, *Cancer Chemotherapy Reports*.

During calendar year 1971 materials for screening were obtained through a variety of mechanisms, including direct purchase, synthesis contracts, natural product development contracts and voluntary submissions. The total number submitted, 15,584, does not reflect as yet the program expansion currently in progress. The input and output of the program for the past six years are shown below:

Input and Output of Drug Development Program

Materials submitted	Calendar year					
	1966	1967	1968	1969	1970	1971
Synthetic and crystalline natural products	8008	7413	10,915	9820	8521	10,151
Crude fermentation products	4284	4982	4256	2184	2172	1215
Crude plant products	--	6253	4245	5109	3499	3015
Animal products	3620	446	641	757	587	1203
TOTAL	15,912	19,094	20,057	17,870	14,779	15,584
IND applications filed	3	4	5	5	6	8

Levels of biological screening during 1971 did demonstrate an increased effort, on the other hand, partially in anticipation of the increased future input but also to relieve a backlog in testing that had developed. Comparison of screening tests for the past four years is shown below:

Screening Tests

	Calendar year			
	1968	1969	1970	1971
L1210 Lymphoid Leukemia	107,445	80,545	79,892	102,424
P388 Lymphocytic Leukemia	14,903	43,223	42,270	53,449
Walker Carcinosarcoma 256	44,563	20,328	10,247	4,368
B16 Melanoma	286	1,240	1,342	1,423
Lewis Lung Carcinoma	244	925	3,033	2,803
Other Tumors	774	426	504	1,780
Total In Vivo	168,215	146,687	137,288	166,247

During 1971, the Chemotherapy Program filed eight IND applications with the Food and Drug Administration, signifying the initiation of clinical studies. The eight materials are:

Methyl-CCNU
1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester) hydrochloride
5-Azacytidine
Ifosfamide
Platinum, diamminedichloro-, cis-
ICRF-159
Rifamycin SV
9H-Purine-6-thiol, 9-β-D-arabinofuranosyl-

This number, compared with the 3-5 applications filed annually during the previous five years, may reflect the effort to accelerate the development of new agents from discovery to clinical trial, or the higher acquisition rate during 1967 and 1968. In any event, it is clear that an increased number of clinical drugs in the future will require increased levels of acquisition and screening. Toward that end, efforts are currently in progress to increase the acquisition rate to 30,000 materials and the screening level to 320,000 tests annually.

The continued acquisition of large numbers of synthetic compounds in sufficient quantity for in vivo screening may prove to be the limiting factor governing the rate of new drug development, and efforts are, therefore, underway to develop and evaluate a variety of cell-free and whole cell in vitro approaches, in the hope that some of these methods may correlate with in vivo screens.

In addition to the studies on in vitro methods, research and development projects of DR&D encompass a wide range of activities, only a few of which can be mentioned in this brief summary. One area of major interest is that of structure-activity relationships. Multiple regression analyses with the nitrosoureas have suggested that more water soluble analogs should be more active and less toxic, and efforts are currently underway to synthesize selected target compounds. Efforts are also continuing in the major areas of nucleosides and folic acid antagonists with the objective of defining more precisely the structural parameters required for activity.

The file of chemical agents has grown to about 160,000 different materials. In order to carry out the substructure searches needed for structure-activity studies, and to simply manage the massive amounts of information involved in daily operations, efforts have been initiated to develop a sophisticated, computerized chemical information system, including a complete system for monitoring compounds from receipt through completion of testing, as well as the mechanization of many manual operations not feasible to continue in an expanded program.

In the area of in vivo screening, studies have continued on the B16 melanoma and Lewis Lung carcinoma systems as possible predictors of activity for human solid tumors. Kinetically, both possess characteristics worthy of further investigation, and it remains to be seen whether they will indeed be useful. Research is also in progress with the spontaneous AK lymphoma and several experimental mammary tumors, all with the objective of finding better systems for predicting drug effects against specific types of human tumors.

Staff and Organization

The DR&D laboratory and contract activities during FY 1972 were carried out by a full and part-time staff of 118: 27 senior professionals (GS-13 or higher), 29 junior professionals, 5 guest workers and fellows, 14 laboratory technicians and support personnel, and 43 clerical and secretarial personnel. The present organization is presented below:

Drug Research and Development

Associate Scientific Director for Drug Research and Development - Saul A. Schepartz

Associate Chief for Laboratory Research - Abraham Goldin
Mammalian Genetics and Animal Production Section - Samuel M. Poiley, Head

Drug Development Branch - Harry B. Wood, Jr., Chief

Chemical and Drug Procurement Section - Robert R. Engle, Head
Chemical and Drug Records Section - Robert B. Ing, Head
Clinical Drug Distribution Section - J. Paul Davignon, Head
Natural Products Section - Jonathan L. Hartwell, Head

Drug Evaluation Branch - John M. Venditti, Chief

Biochemistry Section - Florence R. White, Head
Immunity & Immune Response Modifiers Section - Marilyn Gaston
Screening Section - Betty J. Abbott, Head

Program Analysis Branch - Barbara R. Murray, Chief

Literature Research Section - Dorothy Algire, Head
Publications Section - Pamela Hutchins, Acting Head
Section on Data Processing and Computer Systems Development - Sidney Richman, Head
Scientific Records Section - George F. Hazard, Acting Head

The reports of each of the Branches and Sections which follow will present in great detail the accomplishments and future directions in each area.

Publication (Associate Scientific Director for Drug Research and Development)

Schepartz, S. A.: Screening. Cancer Chemotherapy Reports, Vol. 2, Part 3: 3-8, 1971.

Serial No. NCI 3575

1. Office of Associate Scientific Director, Drug Research and Development, Chemotherapy
2. --
3. NCI - Building 37 and Kenwood Laboratories Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chemotherapy research including fundamental biological and biochemical investigations of antitumor agents

Previous Serial Number: Same

Principal Investigator: Abraham Goldin

Co-Principal Investigators: A. W. Schrecker, J. M. Venditti

Other Investigators: M. Straus, T. Brescia, R. Kojima, V. Subba Reddy, A. Miyakawa, A. Goldberg, D. Houchens

Cooperating Units: G. Cudkowicz, State University of New York at Buffalo; J. M. Venditti and staff, Drug Evaluation Branch, NCI; H. B. Wood, Jr., R. R. Engle, J. Driscoll, Drug Development Branch, NCI; N. Mantel, Biometry Branch, NCI; J.A.R. Mead, Experimental Therapeutics, NCI; R. Gallo, Human Tumor Cell Biology Branch, NCI; M. Sporn, Carcinogenesis, NCI; P. Carbone and E. Henderson, Medicine Branch, NCI; Y. Ikawa, Viral Leukemia and Lymphoma Branch; I. Kline, S. Vadlamudi, and V. Waravdekar, Microbiological Associates, Inc.; A. DiMarco, Farmitalia, Milan, Italy; E. Frei, III, E. Freireich and T. L. Loo, M. D. Anderson Hospital and Tumor Institute; L. Israel, Centre Hospitalier Universitaire Lariboisiere, Paris, France; E. Bonmassar, University of Milan and University of Peruggia, Italy; A. Nicolin, University of Milan, Italy; S. Garattini, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy.

Man Years

Total: 8-1/3
Professional: 5
Others: 3-1/3 (plus animal care support from Mammalian Genetics and Animal Production Section, DR&D)

Project Description:

Objectives:

To conduct fundamental investigations of potential and established clinical cancer chemotherapeutic agents; to obtain biological and biochemical knowledge concerning chemical agents capable of affecting malignant growth; to determine means for improvement of therapy with existing drugs; to investigate the influence of structural modification of drugs on the mechanism of antitumor action; to provide basic information of pertinence to the clinical use of such agents; to study specific problems encountered during the use of these agents in the clinic.

Specific objectives include:

I. Investigations of primary and secondary screening procedures and methodology employed in drug evaluation.

   A. Investigation of characteristics of ongoing screens and drug evaluation systems and of new test systems which may broaden the base of predictability of drugs for clinical activity. Emphasis is placed on predictability systems for clinically useful compounds which were not predicted by the standard screen and systems for the selection of new and novel structures which may have potential in clinical chemotherapy.

II. Investigation of the influence of drugs on tumor cells.

   A. Detailed investigation of mode of action of drugs including purine and pyrimidine antagonists, folic acid antagonists, alkylating agents, natural products, etc.

      1. Determination of the extent and duration of drug action.

      2. Study of the influence of drug on the mitotic cycle.

      3. Study of the lethal action on tumor cells.

      4. Investigation of problems related to drug resistance.

      5. The influence of chemical agents on metastasis and tumor cell sequestration including intracranial involvement.

      6. Studies of drug transport.

      7. Investigation of the sites of action of drugs.

      8. Study of the influence of drugs on the hematopoietic and tissue components of the host in relation to antitumor activity.

      9. Inhibition studies of RNA-dependent DNA polymerase.

B. Investigation of factors which may influence drug action in relation to improvement of therapy.

III. Study of the influence of drugs on tumor-host interactions.

A. Investigation of the basis for drug specificity.

1. Exploration of the influence of novel schedules.

Investigation of the underlying basis for drug scheduling and combination chemotherapy.

2. Detailed studies in inhibition analysis.

a. Determination of metabolite-antimetabolite interrelationships for folic acid congeners, purine antagonists, etc.

b. Investigation of the inhibition of de novo synthesis, lethal synthesis, feedback inhibition, etc.

c. Investigation of collateral sensitivity as a phenomenon which may lead to improved therapy.

B. Exploration of means for augmentation of the host contribution to chemotherapy.

C. Chemotherapeutic investigations on tumor cell kinetics with a view to the total eradication of the tumor cell population in the host.

IV. Antiviral chemotherapy.

A. Study of the action of drugs on virus-induced tumors.

V. Exploration of new and potentially useful biological and biochemical methodology for prediction, evaluation and improvement of drug therapy.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute.

In addition to the laboratories in our building, the Kenwood Laboratories of Microbiological Associates staffed by Microbiological Associates professional and technical personnel serve as a collaborative laboratory resource for the research staff of this program. The chemotherapy research program collaborates closely with the ongoing programs in drug evaluation, drug development, immunology, organic chemistry and pharmacology. The program serves as a focal point for the interdigitation of chemotherapy research including the DR&D screening, developmental and basic chemotherapeutic investigations with collaborating DR&D contractors, and clinical programs of NCI and other institutions. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes, both in the United States and abroad in areas pertinent to chemotherapy.

Since the untimely death of Dr. Jack Glynn, A. Goldin in collaboration with Dr. J. M. Venditti has taken the responsibility for the research program of the Immunotherapy and Immuno-Response Modifiers Section of the Drug Evaluation Branch, including the research of the Immunology Division at Microbiological Associates, Inc.

Methods Employed: Standard laboratory procedures were employed.

International Activities Including Surveillance for New Agents

An important function of this office involves the maintenance of close relationships with world-wide cancer chemotherapy programs with a view to (a) information exchange pertaining to latest scientific developments in chemotherapy, (b) for the opening of new avenues of procurement of synthetic agents and natural products for screening and (c) for the identification of new compounds of potential interest for chemotherapy.

In the above, close liaison is being maintained with investigators in leading institutions, including universities, medical schools and pharmaceutical houses abroad. Active contact continues to be maintained through the Chemotherapy Committee of the International Union Contra Cancrum, the International Agency for Research in Cancer of the World Health Organization, Lyon, France.

In order to provide a more formal and extensive liaison relationship in Asia and Europe, liaison offices have now been established at the Cancer Institute in Tokyo, Japan, and at the Institut Jules Bordet in Brussels, Belgium. It is considered that these liaison offices will provide a mechanism for close collaboration, encompassing both the preclinical and clinical Cancer Chemotherapy Programs of Asia and Europe with that of the National Cancer Institute. The liaison offices will function to gather and exchange preclinical and clinical scientific knowledge in the further development of cancer chemotherapy. They will increase the input into the Chemotherapy Screening Program at the National Cancer Institute and foster collaboration in the study of new anticancer drugs. Through the liaison offices there will be close contact with universities, medical schools, chemical industry and pharmaceutical houses in the various countries. They will provide a means for the continuous review, abstracting and translation (where necessary) of foreign reports.

The two institutions chosen for the establishment of the liaison office are focal centers for cancer research. The Japanese Cancer Institute in Tokyo is the center for the Japanese Foundation for Cancer Research. The Institut Jules Bordet in Brussels is the headquarters for the European Organization for Research in the Treatment of Cancer (E.O.R.T.C.). Both liaison offices have now become functional. The Japanese are currently circularizing research organizations throughout Japan particularly in the commercial field recommending close collaboration and an increase in input of samples for our screening program. They are also in the process of building a new building which will serve as an "Anticancer Drug Evaluation Center" through which it is intended to have further collaboration with us. Their target date for

completion of this center is 1973.

In both Japan and Europe progress is being made in the utilization of quantitative standard screening and drug evaluation protocols, which are similar to those employed in our own program. The leukemia L1210 system has already been introduced into Japan and they have now been supplied with leukemia P-388. It is also planned to send them the Lewis lung tumor, B16 melanoma and other tumors of interest. Similarly the L1210 system is being employed at the Mario Negri Institute in Milan in association with the E.O.R.T.C. and the liaison office, and discussions are being held concerning the further introduction of standardized tumor systems in Europe.

Visits were made by A. Goldin to the following:

1. As part of the liaison program, a visit was made to the Institut Jules Bordet, Cancer Center of the University of Brussels, Department of Medicine and Clinical Investigation under the direction of Professor H. J. Tagnon, Head of the service of Internal Medicine. Discussions were held with Drs. D. M. Staquet and Y. Kenis concerning the liaison program.

2. Eindhoven University of Technology, the Netherlands, in the department of Professor C. Koningsberger where discussions were held with Dr. E. Godefroi, Associate Professor of Organic Chemistry and Head of the Synthetic Research Group concerning submittal of new synthetic agents.

3. Attendance at the 7th International Congress of Chemotherapy in Prague, Czechoslovakia provided a good opportunity for an overview of ongoing research programs and advances in world-wide activity in chemotherapy. A. Goldin (Bethesda) and V. Pujman (Prague) organized and chaired a panel on the "Predictability of Experimental Test Systems for Clinical Chemotherapy" at the International Congress in Prague.

4. Visits to the Mario Negri Institute and the Pharmacology Department at the University of Milan Medical School for discussion of ongoing research programs and collaborative investigations. Discussions were held with Drs. Federico Spreafico, Enzo Bonmassar and Angelo Nicolin and staff at the two institutes.

5. Participation at the International Symposium on Adriamycin at Farmitalia in Milan.

6. Participation as a member of the faculty in a post-graduate workshop course in Curacao, the Netherlands Lesser Antilles in a program organized by the International Union Against Cancer in conjunction with the Antillian Foundation for Medical Education, Curacao and the State University of Groningen (the Netherlands). This course was organized to bring to biomedical scientists and clinical investigators in the various islands of the Carribean and Central and South America, information concerning the latest developments and techniques in pre-clinical and clinical cancer chemotherapy. The course was intended to provide a mechanism for establishing continuing relationships with the

individuals attending and the programs of the various countries. The faculty was international in make-up and the student body represented a number of countries including Argentina, Brazil, Colombia, Costa Rica, Dominica, Guyana, Jamaica, Nicaragua, Puerto Rico, the Dominican Republic, Republic of Panama, El Salvador, Surinam, Trinidad, Venezuela, and the Netherlands Antilles.

7. Visit to the National Cancer Institute at Bogota, Colombia. In addition to a tour of the facility, conferred with Dr. Mario Gaitan-Yanguas, Director of the Institute; Dr. Manuel Iregui Hernandez, Head of Internal Medicine; and Dr. German Jordan, Head of the Gyneocological Service and staff. This group is much interested in furthering their relationship with us in cancer chemotherapy both at the preclinical and clinical levels and discussions were held in this area.

8. Attended a meeting of the Preclinical and Clinical Pharmacology Cooperative Group of the E.O.R.T.C. and participated in the annual assembly of the E.O.R.T.C. at the Institut Jules Bordet in Brussels where a symposium was held on "Aspects of Immunology in Cancer". An opportunity was afforded to review our current preclinical program for the E.O.R.T.C.

9. With S. Carter, visited the new Liaison Office at the Cancer Institute in Tokyo to meet with various Japanese investigators and to review the status of new chemotherapeutic agents and drug trials in Japan. Meetings were held with Professor Tomizo Yoshida, Professor Yoshio Sakurai and their staff concerning the functions of the new Liaison Office and further plans for the new buildings that are being constructed by the Institute. In addition, a two day U.S.-Japan joint seminar on "Anticancer Drug Evaluation" was held at the Cancer Institute in Tokyo. Additional visits were made to the Center for Adult Diseases, Osaka; Takeda Chemical Industries, Ltd., Osaka; the Aichi Cancer Center in Nagoya; and the Tokyo Cancer Center.

10. Attendance at the International Cancer Conference and 8th International Pigment Cell Conference in Sydney, Australia held under the auspices of the International Union Against Cancer and the Australian Cancer Society. In addition to participation at the conference, discussions were held with Drs. C. C. J. Colvenor and J. Loder of the C.S.I.R.O. Division of Applied Chemistry concerning the submittal of compounds and plant products for testing in our program.

11. Participation in U.S.-Australian Conference on Current Cancer Research in Melbourne, Australia. Served as one of the organizers of the conference in addition to presenting a paper. Subject areas included immunology, chemotherapy, immuno-chemotherapy, and tumor viruses.

12. Visit to Dr. B. F. Cain of the Auckland Division Cancer Society of New Zealand concerning compounds he has submitted to our screen which are active and are of potential clinical interest.

Based on original discussions with Professor L. Larionov of Moscow, followed by additional discussions at the meetings in Prague and Curacao plus a series of published articles on the subject, it has been proposed that an international conference on cancer chemotherapy screening and evaluation be held. The subject matter of such a conference could include international cooperation and the development of standards, choice of test systems and interchange of tumor materials of test agents and results of screening and evaluation, the relevance of preclinical screening and evaluation programs to the clinic, in the international chemotherapy effort, could receive prime attention. At a time when great impetus is being given on a world-wide basis in obtaining a cure for cancer, such a conference would appear to be most desirable.

The following talks describing various aspects and results of our program were given abroad during the year by A. Goldin:

1. Predictability of Experimental Test Systems for Clinical Chemotherapy: Summary of the Panel. Presented in Prague (A. Goldin and V. Pujman).

2. Some Factors Influencing the Chemotherapeutic Effectiveness of Adriamycin. Presented at International Symposium on Adriamycin at Farmitalia, in Milan.

3. Anticancer Screening. Presented at Advanced Course in Cancer Chemotherapy, Curacao.

4. Combination Chemotherapy. Presented at Advanced Course in Cancer Chemotherapy, Curacao.

5. Review of Current Developments with Chemotherapeutic Agents at Chemotherapy, NCI. Presented at Clinical Pharmacology Cooperative Group of the European Organization for Research on Treatment of Cancer, Brussels.

6. Current Status of the Preclinical Drug Evaluation Program with Reference to New Drugs of Interest. U.S.-Japan Joint Seminar on Anticancer Drug Evaluation, Tokyo.

7. Closing Address - U.S.-Japan Joint Seminar on Anticancer Drug Evaluation, Tokyo.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

Continued emphasis is being placed on an ongoing program relating structure to antitumor activity in collaboration with Drs. Wood, Engle, Driscoll, Mead and Venditti. Areas covered to date include the folic acid antagonists, purine and pyrimidine nucleosides, methane sulfonates, and benzoquinone derivatives. New drugs continue to be synthesized under the direction of the Drug Development Branch based on structure-activity analyses.

Structure-activity studies include:

A. Preparation and Antitumor Activity of Derivatives of 1-phenyl-3,-3-dimethyltriazene. (Y. Lin, T. L. Loo, S. Vadlamudi and A. Goldin)

A number of 5-triazeno imidazoles are active against experimental tumors. Of these 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (DIC) is clinically useful for the induction of temporary remission in malignant melanoma. Antitumor activity has also been observed in several derivatives of phenyltriazene. In order to extend these observations and to elucidate the structural requirements for antitumor activity in the triazenes and specifically to ascertain whether an imidazole ring with a carboxamide moiety ortho to the triazeno side chain is indispensible in DIC, a series of six derivatives of 1-phenyl-3,3-dimethyltriazene were synthesized.

The 1-phenyl-3,3-dimethyltriazene derivatives were as active as DIC. At least one of the compounds may have a more favorable therapeutic index than DIC.

The studies indicate that the carboxamide group in the phenyltriazenes may be replaced with a carbomethoxy group with no adverse effect. In addition the orientation of either the carboxamide or the carbomethoxy substituent with respect to the dimethyltriazeno side chain is not critical, as all three derivatives, the o- m- and p- showed comparable antileukemic activity. Further, because these phenyltriazenes and DIC were equally active, and moreover, the pyrazole analog of DIC likewise displays antileukemic activity it would appear that in the triazenes the imidazole ring, the phenyl ring, and the pyrazole ring are all essentially equivalent.

B. Some Structure Activity Relationships for Mitomycin Derivatives in the Treatment of Leukemia L1210. (R. Kojima, J. Driscoll, N. Mantel and A. Goldin)

A series of structural analogs of mitomycin C including mitosanes, mitosenes, and N_4-C_{9a} open ring compounds were tested for extent of activity against leukemia L1210. With mitomycin C as a standard, structure-activity analyses were conducted taking into account the toxicity as reflected in optimal dosage and the survival time of the animals. The comparisons were made employing single treatment over a series of dose levels with each drug. Of the compounds tested the two most effective in increasing the survival time of the leukemic mice were mitomycin C and porfiromycin, the optimal dose of the latter being considerably increased. The various structural derivatives showed a wide range of toxicity and the alterations of therapeutic effectiveness ranged from minimal decreases to complete loss of activity The structural substituents in various positions of the mitomycin molecule including the quinone, aziridine and carbamoyloxymethyl moieties appear to be interrelated with respect to resultant effect. The wide range of activity response in relation to structure suggests additional modification and investigation of the mitomycin

class of compounds.

C. 5-Azapurines and the Structures of sym-Triazole Intermediates. (T. Hirata, L. Twanmoh, H. B. Wood Jr., A. Goldin and J. S. Driscoll)

Spectral properties, methods of synthesis and modes of degradation were described for several new members of the 5-azapurine series.

See report of Drug Development Branch, DR&D, Chemotherapy.

D. Folic Acid Antagonists. (J.A.R. Mead, H. B. Wood Jr. and J. S. Driscoll)

Collaborative studies were continued on the modification of structure and resultant antileukemic activity in the folic acid antagonist series. The demonstrated activity of tetrahydrohomofolic acids against leukemia L1210 resistant to methotrexate has now also been observed for 5-methyltetrahydrohomofolic acid (Mead). This observation is of particular significance since the 5-methyltetrahydrohomofolic acid appears to be essentially equivalent in its activity to methotrexate against the sensitive line of leukemia L1210. Systematic studies of a series of halogenated derivatives of methotrexate have interesting observations pertaining to toxicity and antileukemic activity (see annual report of J.A.R. Mead).

II. Mechanism of Action of Antitumor Agents

A. Reverse Transcriptase Inhibitors. (A. W. Schrecker, R. C. Gallo and M. B. Sporn)

The effect of reverse transcriptase inhibitors of potential chemotherapeutic interest on the RNA-dependent DNA polymerase of Rauscher leukemia virus (RLV) has been studied and compared with their effect on DNA-dependent DNA polymerase purified from normal human blood lymphocytes. A series of synthetic thymidylate derivatives bearing reactive substituents on 3'- and/or 5'-phosphate ester groups or on the 3'-position of the deoxyribose moiety was investigated in detail. The most active compound of the series, thymidine-3',5'-di-m-bromoacetamidophenylphosphate (TDMB), inhibited viral reverse transcriptase (rA.dT template) 50% at 50 μM concentration, and 95% when it was preincubated with RLV before addition of rA.dT and TTP. In the absence of preincubation, inhibition was competitive with template. There was some evidence of active-site-directed pseudo-irreversible enzyme inhibition. This series of compounds is not selective because DNA-dependent reactions were inhibited similarly. The work is being continued with the aim of finding more specific inhibitors.

The inhibition of viral and cellular DNA polymerases by arabinosylcytosine 5'-triphosphate (ara-CTP) is at present under investigation. It is known that ara-CTP, the active intracellular form of arabinosylcytosine (ara-C), is a DNA polymerase inhibitor which

competes with the substrate dCTP. Kinetic studies with various purified DNA polymerases, including cell-cycle dependent enzymes from human blood lymphocytes, are being carried out to determine the affinity parameters of ara-CTP and dCTP for these enzymes. A selective inhibition might explain the known clinical effectiveness of ara-C.

III. Drug Evaluation Systems

In a continuation of the analysis of screening systems provided in a chapter for Cancer Medicine by A. Goldin and S. K. Carter, a review article has been written by A. Goldin, S. Carter and N. Mantel "Evaluation of Antineoplastic Activity: Requirement of Test Systems and Statistical Design" for A Handbook of Experimental Pharmacology - Antineoplastic and Immunosuppressive Agents.

This article provides a description and discussion of the linear array, covering selection and acquisition of agents for screening (Stage I) and the determination of drug activity (Decision Network IIA), drug evaluation and development (Decision Network IIB), preclinical toxicology (Decision Network III) and clinical evaluation (Decision Network IV, V, and VI). Also included are discussion of the choice of screening systems and an analysis of principles of screening and drug evaluation. One of the points emphasized is that the labeling of a drug as false positive for a given screening system is dependent on a rigorous definition of what constitutes an adequate clinical trial. On examination of the details of schedule dependency with its experimental and clinical correlations the possibility is presented that very few cancer chemotherapeutic agents have been completely characterized. Similarly, correlations of animal and clinical drug action and effectiveness require characterization of other factors influencing drug effectiveness in animal tumor systems and the clinic. It is emphasized that rigorous employment of current and new model test systems is most important to the discovery and optimal application of new antitumor agents with salutary action against clinical neoplasia.

A. Models for Remission Induction and Remission Maintenance

1. Leukemia P-1798. (S. Vadlamudi, M. Padarathsingh and A. Goldin)

It was previously demonstrated that leukemia P-1798 could serve as a model for studies of remission induction and remission maintenance. In this system the combination of vincristine and prednisone appeared more effective than the drugs employed individually in the induction of remission and in increasing the survival time of the leukemic animals.

An experimental model has now been developed for the study of combination chemotherapy with three drugs in the leukemia P-1798 system. In one study vincristine plus prednisone was employed for remission induction and methotrexate was used for remission maintenance. This therapy provided only a moderate additional increase in overall survival time as compared with that observed for unmaintained remission following remission induction with prednisone plus vincristine or vincristine alone.

2. AKR model for remission induction and maintenance. (A. I. Goldberg, J. P. Glynn, M. Kende, N. Mantel and A. Goldin)

A remission induction-maintenance therapeutic design has been developed employing the AKR spontaneous lymphoma system. This system is being employed to mimic clinical remission induction and remission maintenance protocols. In this system the combination of vincristine plus prednisone induced remission in approximately 60% of the animals. Of the drugs examined cyclophosphamide was the most effective as a maintenance agent, increasing the median survival time by 250% over the placebo maintained groups. The studies are being conducted in coordination with those of the Southern Research Institute and the Ad Hoc AKR Leukemia Committee directed by Dr. Emil Frei, III (see report of Immunotherapy and Immune Response Modifiers Section, Drug Evaluation Branch).

B. The Chemotherapeutic Model with the K-B-N-Cl-6 Non-producer MSV Transformed Cell Line. (T. Brescia, Y. Ikawa and A. Goldin)

The K-B-N-Cl-6 cell line is a "non-producing" MSV transformant of a continuous culture line of a BALB/c mouse embryo and has been recloned in vitro. When the cells are injected into BALB/c mice they develop morphologically into an "adenocarcinoma". The tumor kills the host in 2-3 weeks depending on the concentration of the tumor cell inoculum and the route of administration.

It is considered that the MSV transformed cell line may provide a tumor system analogous to human epithelial tumors for use as a drug screening system. The system approaches a spontaneous tumor in the sense that it is a "non-producer" of viral genome and is not immunologically reactive in the BALB/c mouse. Cyclophosphamide has now been demonstrated to be highly active in vivo against this tumor. Long term survivors have been obtained following single or multiple doses of cyclophosphamide at inoculum concentrations where 100% of the control animals succumbed within 20 days following inoculation. Methotrexate has been essentially ineffective against this tumor as a single treatment or on several multiple-dose schedules. The various parameters and spectrum of drug sensitivity for this MSV in vitro transformed line are currently being explored.

C. Studies on Correlation of In Vitro and In Vivo Effectiveness of Drugs. (S. Vadlamudi, W. A. Thomas and A. Goldin)

This program has been continued in an attempt to establish a battery of in vitro sublines of leukemia L5178Y, leukemia L1210 and other tumors for potential use in screening. A number of lines, including L5178Y, L5178Y/Asparaginase, L1210/6-MP, L1210/BCNU, glioma 29, neuralemmona have now been established. Experiments are in progress to determine in vitro-in vivo correlations with respect to drug effectiveness. It was demonstrated earlier that an L-asparaginase resistant variant of leukemia L5178Y was resistant to L-asparaginase

both *in vivo* and *in vitro*. It is considered that one or more screening systems are necessary for the testing of new agents which are available in very limited supply. This program is an effort in that direction.

IV. Chemotherapy Studies

A. Drug Scheduling

1. Sequential chemotherapy. (M. Straus and A. Goldin)

It was previously demonstrated that early administration of a high priming dose of therapeutic agents such as cyclophosphamide may enhance therapeutic effectiveness when followed by treatment with antitumor agents such as methotrexate, a cell cycle stage specific agent. It would appear in such case that early administration of a high priming dose of cyclophosphamide lowers the tumor cell population sufficiently so that scheduled doses of methotrexate can kill tumor cells more effectively. In further studies with methotrexate in the treatment of leukemia L1210, it was also previously demonstrated that treatment with a relatively high initial dose followed by lower doses resulted in survival-times of the leukemic mice which were longer than those obtained when constant dose levels were employed.

A study has been conducted in which it was demonstrated that employment of a high priming dose schedule of a new drug, carbazilquinone, can also result in increased antileukemic effectiveness. In one experiment, geometrically spaced doses of carbazilquinone were employed at the first treatment and each was followed by various geometrically spaced levels of regularly scheduled carbazilquinone at 4-day intervals. In a number of groups of animals which had received a high loading dose of carbazilquinone followed by lower doses, there were cured animals. None of the mice treated on a constant dose schedule of carbazilquinone were cured. The results of this study lend further support to the concept that the loading dose schedule can be usefully incorporated early in the chemotherapeutic evaluation of new agents. The potential clinical implications are worthy of consideration.

2. Scheduling of methotrexate. (M. Straus and A. Goldin)

Studies were conducted on the effect of the day of dosage omission on the survival time in a regularly scheduled methotrexate treatment of leukemia L1210. In these studies it was demonstrated that, in general, the earlier a dose of methotrexate was omitted the shorter was the survival time of the animal. The results lend support to prior observations that the percentage tumor cell kill by methotrexate may decrease as the population of tumor cells increases. The earlier a dose of scheduled methotrexate is omitted the greater are the number of subsequent doses which

must act against an increased population, thereby resulting in a decrease in survival time. This study further emphasizes the importance of early administration of an effective priming dose.

B. Schedule Dependent Therapeutic Synergism for L-asparaginase and Methotrexate. (S. Vadlamudi, V. V. Subba Reddy and A. Goldin)

Therapeutic synergism has now been observed over a variety of schedules with the combination of L-asparaginase and methotrexate. The most effective schedule would appear to be one in which a single treatment with L-asparaginase was followed 6 hours later by daily treatment with methotrexate. With all of the effective schedules asparaginase was given as a single treatment and methotrexate was given as a series of treatments. On one schedule of therapy where daily treatment with L-asparaginase was followed sequentially by daily treatment with methotrexate there was an actual loss in therapeutic effectiveness as compared with treatment with either drug alone.

C. Sequential Chemotherapy with Adriamycin and Other Antitumor Agents.

1. Effect of sequential treatment with adriamycin and methotrexate in leukemic mice. (A. Miyakawa, M. Padarathsingh, S. Vadlamudi and A. Goldin)

Because of the limiting toxicity of adriamycin on repetitive administration, attention has been focused on combination chemotherapy. In one study, sequential and alternating schedules of adriamycin and methotrexate were found to be more effective than adriamycin alone or methotrexate alone in increasing the survival time of mice with leukemia L1210. Effective schedules included (a) a single treatment with adriamycin followed sequentially by a series of daily treatments with methotrexate; (b) adriamycin every 3 hours for a single day followed by sequential daily treatment with methotrexate; (c) adriamycin daily for 5 days alternated with methotrexate daily for 5 days. In these studies the optimal dose of methotrexate in the sequential and alternating schedules approximated that observed with methotrexate alone. It is suggested that non-additivity of drug toxicity may provide a basis for therapeutic response for the combination of adriamycin plus methotrexate.

2. Effect of sequential treatment with adriamycin and the imidazole carboxamide derivative (DIC) (NSC-45388). (I. Kline, R. Woodman, R. Cysyk, M. Gang, A. Goldin and J. M. Venditti)

A similar advantage in sequential therapy was observed with a combination of adriamycin plus the imidazole carboxamide derivative (NSC-45388) (DIC) against leukemia L1210. Treatment with adriamycin every 3 hours for a single day followed by daily treatment with DIC was approximately twice as effective as adriamycin alone or DIC alone in increasing the survival time

of the leukemic animals. With this sequential therapy also the optimal dosage for each drug was similar to that observed with the individual drugs suggesting that there was little if any additional toxicity for the combination.

The sequential therapies with adriamycin plus methotrexate and adriamycin plus DIC have stimulated clinical interest.

D. Immunity Plus Chemotherapy

1. Further studies on antigenic changes in L1210 leukemic cells. (A. Nicolin, S. Vadlamudi, E. Bonmassar and A. Goldin)

It was previously demonstrated in this program that the antigenicity of leukemic cells may be altered as the result of treatment with an antitumor agent. Employing the chromium (CR^{51}) release test, specific cytotoxic antibodies have now been demonstrated in sera of mice immunized with cells of a subline of leukemia L1210 resistant to cytosine arabinoside. Although the serum of mice immunized against the cytosine arabinoside resistant subline demonstrated specific cytotoxic activity against the cells of the same resistant subline (ara-C/R) it was essentially inactive against the original sensitive leukemia. Animals immunized against the sensitive leukemia, on the other hand, developed only weak antiserum which did not evidence specific activity.

Enhanced chemotherapeutic activity against resistant sublines of leukemia (collateral sensitivity) has been clearly illustrated in several laboratories. This collateral sensitivity may be the resultant of the combination of increased immunogenicity of the L1210 drug-modified sublines plus chemotherapy, constituting a form of immuno-chemotherapy. The demonstration of specific cytotoxic serum cited above suggests the exclusion of an unspecified increased immunosensitivity of the modified leukemic cells. Since specific cytotoxic antibodies were observed with the resistant subline but not with the sensitive line of L1210 the appearance of increased antigenicity of the drug modified leukemic cells would appear to be attributable to the expression of new antigen(s) accompanying the development of resistance.

2. Antigenic changes of L1210 leukemia in mice treated with 5-(dimethyltriazeno)-imidazole-4-carboxamide. (E. Bonmassar, A. Bonmassar, S. Vadlamudi and A. Goldin)

Strong antigenic changes were induced in two L1210 leukemia lines (L1210 Cr and L1210 Ha) after several passages in CDF_1 and BDF_1 mice treated with 5-(dimethyltriazeno)-imidazole-4-carboxamide (DIC). The leukemic cells of both lines originally compatible with DBA/2 and related hybrid hosts elicited a pronounced allograft reaction in the same animals. The L1210 Cr line was

sensitive to the antitumor activity of DIC while L1210 Ha was not. It would appear then that alteration of antigenicity may occur on exposure to drug even where no therapeutic response is elicited. No specific relationship appears necessary between the origin of drug resistance and the appearance of antigenic changes.

E. Increased Lifespan in AK Leukemia Mice Using Prophylactic Cytoxan Chemotherapy. (M. J. Straus, S. C. Choi and A. Goldin)

AKR mice die of spontaneous leukemia at about age 9.5 months. Mice treated prophylactically with cyclophosphamide from age 5 months to 10 months at intervals of 1 week, 2 weeks, or 4 weeks at 120 or 60 mg/kg i.p. (with the exception of cytoxan 120 mg/kg q. 1 week which was toxic) yielded mean survival times of 11.5 months which was significantly greater than controls (9.9 months). The 12-month survival rate for controls was 17% whereas the 12-month survival rate for treated groups was approximately 50%.

V. Collaborative Studies

Collaborative studies with the Drug Evaluation Branch and the Biometry Branch, NCI were continued. (J. M. Venditti and Staff; I. Kline, S. Vadlamudi and Staff; N. Mantel and A. Goldin)

These involve studies on schedules of drug administration, sequential and concomitant combination chemotherapy, studies on resistance and cross-resistance, etc.

Collaborative studies with the Drug Development Branch, NCI have been continued in the area of structure-activity analysis in chemotherapy. (H. B. Wood, Jr., R. R. Engle and J. Driscoll)

Collaborative studies with the Medicine Branch, NCI on preclinical investigation of specific problems relating to the usage of drugs in the clinic. (P. Carbone and E. Henderson)

Significance to Bio-Medical Research and the Program of the Institute

The chemotherapy research program is operated in direct relationship to the needs of the Chemotherapy Program, NCI. The investigations are conducted at the NCI and as part of a collaborative program at the Kenwood Laboratories. The program is conducted in close coordination with the activities of the Drug Evaluation Branch, the Drug Development Branch, the Laboratory of Chemical Pharmacology, and the Medicine Branch of the Cancer Institute. The research is dovetailed with the screening program and contract activities of DR&D.

New methodology is transmitted to the screening program and pertinent basic observations are transmitted for possible clinical application. New drugs of clinical interest are investigated with respect to optimal usage and mechanism of action and recommendations are made to the clinic. Problems arising in the use of chemotherapeutic agents are subjected to additional investigation.

Proposed Course of the Project

The various aspects of the program will be continued and appropriate modifications made in accordance with the laboratory findings and the clinical interests.

Further emphasis will be placed on detailed investigations with leukemia L1210, Lewis lung carcinoma and resistant tumors including characterization of drugs with respect to their action in the mitotic cycle of normal and neoplastic cells. Studies will be conducted on the influence of cell synchrony in chemotherapeutic effectiveness of drugs. Additional tumor screening systems will be investigated with particular reference to drugs of clinical interest which are not active in the L1210 leukemia or Walker carcinosarcoma systems. This includes tumor systems such as P-388, P-1534, P-1798, the LPC1 plasma cell system, transplantable melanoma, viral-induced leukemias and functional and spontaneous tumors. Emphasis will be placed on investigation of systems which may permit screening with limited amounts of test materials.

Studies will be continued on means for estimation of numbers and location of residual sequestered tumor cells and of means for their eradication.

Immuno-chemotherapy studies will be continued. Studies on the influence of drugs in altering tumor cell antigenicity will be extended from the point of view of improvement of therapy. Attention will be focused on the relationship of immuno-chemotherapy to the phenomena of "collateral sensitivity" and "synergism". Studies on the potentiating effects of relatively non-specific weak graft-versus-host reactions will also be investigated further.

Detailed evaluation and investigation of drugs of potential clinical interest will be continued. Additional investigation will be conducted on scheduling procedures which will best permit recommendations for clinical use of new agents. New and novel scheduling methodology will continue to be investigated. Studies will be continued to determine procedures for reducing drug toxicity and improving therapeutic effectiveness. This will include investigation of differential rates of recovery from drug toxicity of host tissues as compared with the tumor cell population. Studies with the spleen colony assay system will be continued as an ancillary index for the determination of optimal scheduling of therapy. Additional studies will be conducted on the maintenance of drug levels in the blood and transport, sites of action, etc. Biological and biochemical investigations will be continued to determine the characteristics and mechanism of action of antitumor agents. Attention will be focused on the phenomena of tumor invasiveness and resistance and potential chemotherapeutic approaches. Studies will be conducted on possible means for creating differential deficiencies of essential metabolite in tumor cells as opposed to vital cells of the host. In collaboration with clinical investigators, biochemical investigations will be extended to human tissues of interest. In relation to clinical interest, emphasis will continue to be placed on studies of remission induction and remission maintenance including intensification, consolidation, reinduction, etc. This will include study of suitable systems, characterization of drug action and factors which may influence the effectiveness of drugs in various aspects of remission induction and remission maintenance. Studies with the AK and P-1798

leukemia systems will be continued in this regard. In view of the strong clinical interest in multiple drug therapy emphasis will be placed on further investigations of combination chemotherapy with two or more drugs and on adjuvant chemotherapy.

Various facets of programs in progress and problems of particular clinical interest will be investigated. Increasing attention will be focused on preclinical investigations in liaison with clinical investigators.

Honors and Awards: A. Goldin received the Purkinje Award, Prague, Czechoslovakia.

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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR D R & D

INTRODUCTION

During 1971, as in prior years, the Mammalian Genetics and Animal Production Section provided supportive services for intramural as well as collaborative program areas of the NCI. These activities included the provision of a variety of strains and stocks of laboratory animals, maintenance of disease diagnostic laboratories, and the furnishing of informational assistance to users as well as producers of laboratory animals.

The MG & APS's overall program increased in scope and dimension during this period. The base of resources was expanded and refinements in depth were implemented. Events during the past year included the development of additional clean colonies and an acceleration in the emergence of colonies with defined microbial flora.

The Section's animal production activities included the primary isolator colonies, animals reared within the confines of bio-containment environments, genetically defined animals reared in modified conventional environments, rodent production centers, and large-scale production of hybrids of inbred animals as well as the propagation of parental strains of inbred mice. Ancillary projects included diagnostic laboratories; the development of additional standards for the care and maintenance of laboratory animals; identification of the normal intestinal flora of laboratory rodents; conferences on the application of laboratory animals in biomedical research and improvements in animal quality; development of professional and sub-professional training programs; and an expansion of the DR & D accreditation program. The Section continued to furnish animal care services for the Chemotherapy area in Building 37.

The Section is called upon for assistance in the design of animal facilities; development of training programs; participation in training programs; the location and availability of animal stocks and strains; assistance in diagnosing unusual conditions in animals; and information of general interest. Staff members participate in seminars and symposia; serve as members of regional, national and international committees; serve as instructors in and evaluators of training programs; and as members of committees in scientific societies. In-house research includes the effects of diets upon reproduction and growth, the influence of environmental factors upon the reproductive characteristics of animals, and the development of remedial systems for problems in animal health. The results of these studies are published in scientific journals.

During 1971, the Section provided 71 kinds of animals of eight mammalian species. The major portions consisted of inbred and hybrids of inbred mice. All colonies were funded by contract mechanisms, and, with the exception of beagle hounds and F344 rats, all breeding animals were furnished by the Section. Small groups of animals were supplied to the tumor repositories for the maintenance of tumors and as hosts for tumors shipped to screening

laboratories.

Section staffing consists of one senior staff member, three professional associates, one animal contracts assistant, one animal caretaker supervisor, and four animal caretakers.

The Senior staff member and the three professional associates served as Project Officers and/or Assistant Project Officers for the following contracts:

BDF_1 hybrid mouse production	12
CDF_1 hybrid mouse production	9
Inbred mouse production	6
BALB/c production	1
Inbred rat production	2
Beagle hound production	3
Genetic-production centers	6
Germfree inbred rat production	1
Primary genetic-production centers	2
Disease Diagnosis and research	3
Histocompatibility studies	1
Testing and research	3
Standards for Animal Care and Maintenance	1
Conferences on Laboratory Animal Medicine and Users of Laboratory Animals	1
	51

The senior staff member also serves as ex-officio Assistant Project Officer for advice/guidance in animal husbandry practices for all contract-supported testing and research laboratories.

ACCOMPLISHMENTS

Animal Production

The expansion of the NCI's studies during the last six months of 1971 created a need for additional animals. Program changes included expanded efforts in the areas of compound screening, chemotherapy of spontaneous leukemia in AKR mice, and the treatment of mammary tumors in C3H/He mice. Animal Production activities were accelerated for DBA/2, C57BL/6, AKR (weanlings and aged mice), C3H/He, BDF_1 hybrids, and CDF_1 hybrids. Hamster usage was confined almost entirely to special biochemical studies. Beagle hound requirements for toxicology increased approximately 40%. The overall use rate of all species during 1971 exceeded the number used in 1970 by approximately 500,000 animals (Table I - Appendix). These results are in contrast to the latter period which witnessed a decrease of approximately 65,000 animals when compared to the number furnished in 1969.

As in the past, the major portion of the animals, in terms of volume, originated from colonies supported by fixed price contracts. These organizations furnished DBA/2, C57BL/6, BDF_1 and CDF_1 hybrids, Fischer 344 rats and beagle hounds.

Genetic-Production Centers

These centers were expanded in order to produce additional breeders for hybrid production, and to expand the strains and stocks of rodents which were required for the various NCI program areas. Although the profile of strains, stocks and species were essentially unaltered, production levels were increased or decreased, dependent upon laboratory use rates. These sites serve as sources for unusual hybrids which are developed for very specific investigations. Examples are BAF_1, CBF_1, $AKD2F_1$, $D2AKF_1$, and $NZWxNZBF_1$ hybrids. The quantities required are generally comparatively small, and they are discontinued when the studies have been completed.

The Genetic-Production Centers also supply breeding animals for contracts in foreign countries, and for collaborative studies between scientists in other countries and chemotherapy staff. During 1971, animal shipments were made to Belgium, Italy, India, Japan, Australia, England, Wales, Brazil, France, Canada, Republic of the Philippines and New Zealand. Breeding starts of unusual strains were also provided to various research laboratories in the United States.

During this period animal husbandry practices were upgraded, and physical plants were renovated. Advanced methodology included improved air handling systems, acquisition of improved sanitizing equipment, personnel training programs, and increased preoccupation with improvements in animal health.

Primary Genetic-Production Centers

These centers include the use of flexible film isolators for the re-derivation of animals. The technology associated with the use of this equipment furnishes a means for the elimination of potential pathogens and other microbial forms which interfere with the orderly functioning of testing and research activities. During 1971, four additional rat strains and six other mouse strains passed through this system and have been established in biocontainment environments. The latter areas were expanded in order to furnish clean breeding starts for Rodent-Production Centers.

The combining of the inbred mouse, hamster, inbred rat and outbred rat germfree contracts with the ARS/Sprague-Dawley Co. into one inclusive contract provided a greater flexibility and resulted in increased efficiency for these activities.

The Tulane School of Medicine center maintained eleven inbred rat strains in the germfree state. In addition, the contractor established six strains in room environments for the production of limited numbers of rats for special projects.

The Primary Center managed by the Charles River Breeding Laboratories (CRBL) reached full operational capacity during the third quarter of 1971. This contract possesses unique capabilities for the development of the zonal and modular concepts for animal colony management. Portions of buildings are enclosed by partitions to form an autonomous zone. These zones do not share supporting services with other areas. Portable laboratories are installed

in other locations on the CRBL site as individual modules to form an isolated complex. Each module is maintained as an entity.

Zonal and modular configurations provide an ideal building system for maximum control of environmental factors. This past year's experiences have convinced us that this is the route to follow in the future. The ARS/Sprague-Dawley and Charles River Breeding Laboratories systems have furnished mice, rats and hamsters for long-term carcinogenesis studies. Reports from the laboratories involved indicate that the animals have not demonstrated the presence or the effects of pathogens, and have remained parasite-free for the duration of the studies.

Rodent-Production Centers

These centers served to produce large numbers of inbred mice, and for the aging of AKR mice. The animals furnished to these centers have, in general, originated from environmentally controlled colonies. These centers were either constructed or renovated during the early part of 1971. Their designs were based upon the dual corridor system and they were furnished with modern equipment. The quality of the animals produced in these facilities was superior to those bred in conventional centers.

In-House Animal Colony Activities

Section staff continued to provide animal care for in-house Chemotherapy studies in Building 37. The caretaker staff gained an additional position in order to keep abreast of the ever-increasing workload.

Service Laboratories

The disease diagnostic service laboratories at the Battelle Memorial Institute, IIT Research Institute and New Jersey College of Medicine and Dentistry maintained active roles in our quality control program. The workscope of each contract was expanded in direct ratio to the expansion of the production colonies and testing and research laboratories. Each service laboratory received an increased number of samples for diagnosis and participated in additional troubleshooting activities. They were of exemplary assistance in the evaluation of a presumptive outbreak of anthrax. It was eventually found that the original diagnosis by a consultant for the contractor was in error. Our laboratories' findings were confirmed by the CDC and FDA. Intermittent minor events were carefully diagnosed and, with the institution of remedial measures, were quickly brought under control.

The Mouse Pox Service Laboratory continued its function of supplying vaccine for protection against Ectromelia throughout the scientific community. We did not receive reports from any institution concerning the presence of Ectromelia.

Salmonella positives during 1971 continued below 1% throughout the year. Only one incident of S. typhimurium was reported. The balance of the few positives received represented unusual serotypes such as S. anatis, S. infantis, S. blockley, etc.

The most prevalent viruses detected during this period included Sendai and PVM. LCM was detected in a commercial colony. We persuaded the colony management to euthanize the animals and fumigate the infected rooms. After this was accomplished, we provided breeding replacements from MG & APS colonies. Subsequent testing of the reconstituted strains demonstrated that the area remained free of LCM.

Health Research, INC., continued its studies of the histocompatibility relationships among sublines of the mouse strains maintained by MG & APS in the various centers. Skin grafts exchanged between these mice and counterparts from the NIH and the Jackson Laboratory demonstrated that detectable subline drift did not occur. This laboratory also began its studies of the karyotypes of the tumors used in the DR & D screening program.

The Mason Research Institute continued its routine surveillance of the genetic characteristics of the inbred strains of rats maintained in the genetic centers. In addition, the program to identify the Hag antigens of inbred rat strains was expanded with the inclusion of eight additional strains.

The incidence of dead and ill animals reported by consignees during 1971 was 2.99%, an increase of 0.43% in comparison to 1970. This slight increase was anticipated. Minor problems generally occur when a program is expanded, for it involves new producers and additional laboratory contractors, and both of these groups require several months to stabilize procedures.

The Accreditation Program

The DR & D scheme for the accreditation of suppliers or potential suppliers of animals for testing and research activities once again demonstrated its value as a facet of the quality control program. A minimum of two inspection visits was made to each facility by Section staff members during the year.

Non-routine visits were made to previously unaccredited companies who had responded to notices in the Commerce Business Daily. The majority of these organizations were determined to be acceptable and were forwarded RFP's. Several had been visited in the past, had effected required improvements, and were accredited. On the other hand, others had become lax in their methods and were removed from our listing of satisfactory potential sites for contract production programs.

During 1971, visits were made to production and research animal colonies as follows:

Genetic Production Centers	31
Primary Genetic Centers	12
Rodent Production Centers	15
Beagle Production Colonies	6
Inbred Mouse Production Colonies	5
Inbred Rodent Colonies	24
Hybrid Mouse Production Colonies	60
Research Animal Colonies	43

Service Laboratories	9
Miscellaneous visits	82
	287

Task Order 6 with the National Academy of Sciences developed the following standards:

Standards and Guidelines - Swine
Guide to Infectious Diseases of Mice and Rats
Laboratory Animal Literature (LAMESH)
Standards and Guidelines - Dogs

The efforts of the ad hoc committees which develop these standards have produced valuable information for producers and users of laboratory animals. As a consequence, they have been incorporated into training programs, and are used as standard protocols by procurement officers in laboratories and academic institutions.

Task Order 16 with the National Academy of Sciences arranged the following conferences during 1971:

A Guide to Postdoctural Education in Laboratory Animal Medicine
The Future of Laboratory Animal Research and Resources Programs
Animal Models for Biomedical Research IV
Defining the Laboratory Animal
Symposium on Diseases of Laboratory Animals Complicating Biomedical Research

The conferences sponsored by this Task Order provide opportunities for all scientists in the biomedical field to discuss mutual problems and develop solutions. Opportunities are also made available for discussions concerning improvements in laboratory animal medicine; explorations in future approaches to specific areas of research; identify animal counterparts for human conditions; and discuss the use of unusual animal species for investigations.

Technical Assistance

The Section responded to a variety of requests for assistance in building design, diagnosis of diseases, assistance in correcting pathologic conditions in animal colonies, program planning, the development of methodology for the care of animals in long-range studies, and the development of training programs. Staff members were requested to present papers at regional and national scientific meetings.

The MG &APS and the Chemotherapy exhibits were presented during the annual meetings of the American Veterinary Medical Association and the American Association for Laboratory Animal Science. The Section received the annual award for the outstanding exhibit from the latter organization.

Proposed Course of Project

The Section's program for continued improvement in animal quality will be expanded. Efforts will be directed towards prevention rather than the cure of abnormal conditions. Preventive programs will be reliant upon expansions of isolator systems for the propagation of breeder producing colonies with defined microbial flora. This phase of the activity will furnish breeders for bio-containment colonies. These closely controlled colonies will be located in facilities specifically designed for these purposes. Contractors will be encouraged to construct zonal and modular buildings for the propagation of super-clean colonies.

Current projects to define biological and physiological parameters for genetically characterized rodents will be expanded to include additional strains. Contractor's staffs will be advised of advancements in the field of animal production and encouraged to participate in training courses and seminars.

It is anticipated that the NCI Fort Detrick Center will become operational during 1972. The Section is looking forward with anticipation to the opportunity to implement and evaluate newer concepts for the production of clean animals for long-term studies.

TABLE I

ANIMALS SUPPLIED DURING CALENDAR YEARS 1970 AND 1971

Inbred Mice	1971	1970	+Increase -Decrease
A/HeCr	9,826	10,236	- 410
A/JCr	1,769	1,883	- 114
AKR/Cr	201,239	123,741	+ 77,498
-pregnants	6	4	+ 2
AL/NCr	1,764	483	+ 1,281
-pregnants	8	--	+ 8
BALB/cCr	134,458	153,050	- 18,592
-pregnants	11,897	10,501	+ 1,396
-litters	265	164	+ 101
BALB/cJCr	256	306	- 50
B10.A/Cr	1,674	2,181	- 507
B10.129(5M)/Cr	319	1,799	- 1,480
B10.A(2R)/Cr	310	2,164	- 1,854
B10.A(5R)/Cr	3,590	2,281	+ 1,309
B10pd/CzCr	641	1,707	- 1,066
BRVR/Cr - litters	5	--	+ 5
BRVS/Cr	58	--	+ 58
BSVS/Cr	38	4	+ 34
-litters	12	--	+ 12
CBA/JCr	3,714	1,922	+ 1,792
-pregnants	9	--	+ 9
CFW/PlCr	1,215	171	+ 1,044
C3H/BiCr	139	165	- 26
-pregnants	2	--	+ 2
C3H/HeCr	47,813	28,309	+ 19,504
-pregnants	38	15	+ 23
C3Hf/HeCr	3,456	3,128	+ 328
C57BL/6Cr	113,553	91,623	+ 21,930
-pregnants	164	76	+ 88
-litters	8	5	+ 3
C57BL/6KaCr	2,510	2,422	+ 88
-pregnants	6	21	- 15
C57BL/10ScCr	2,885	3,201	- 316
C57L/Cr	996	806	+ 190
DBA/1J	--	20	- 20
DBA/2Cr	98,584	99,516	- 932
-pregnants	--	6	- 6
-litters	14	10	+ 4
I/Cr	67	--	+ 67
NIH/PlCr	1,413	1,289	+ 124
-pregnants	--	30	- 30
NZB/Cr	3,329	930	+ 2,399
-pregnants	--	5	- 5
-litters	12	1	+ 11

TABLE I
(Continued)

	1971	1970	+Increase -Decrease
Inbred Mice			
NZW/Cr	1,842	1,786	+ 56
-litters	7	1	+ 6
PRI/PlCr	2,723	2,419	+ 304
SJL/JCr	6,595	5,552	+ 1,043
-pregnants	15	--	+ 15
SM/JCr	1,080	978	+ 102
	647,856	544,072	+ 103,784
Pregnants	12,145	10,658	+ 1,487
Litters	323	181	+ 142
Hybrid Mice			
$AKD2F_1$	1,624	2,336	- 712
$ALZBF_1$	--	44	- 44
BAF_1	4,937	4,606	+ 331
BDF_1	1,446,831	1,259,877	+ 186,954
-pregnants	--	6	- 6
$B6AKF_1$	4,916	2,364	+ 2,552
$B6KC3fF_1$	2,654	938	+ 1,716
$B6C3F_1$	16,491	800	+ 15,691
CBF_1	6,674	7,698	- 1,024
$CCEF_1$	31	--	+ 31
CDF_1	570,042	286,744	+ 283,298
-pregnants	3	2	+ 1
$C3ZBF_1$	--	117	- 117
$(C3ZBF_1xNZB)F_1$	--	87	- 87
$D2AKF_1$	3,048	4,140	- 1,092
$D2ZBF_1$	--	65	- 65
$(D2ZBF_1xNZB)F_1$	--	103	- 103
$ZBZWF_1$	--	235	- 235
$(ZBZWF_1xNZB)F_1$	--	246	- 246
ZWZBF	1,543	962	+ 581
-pregnants	140	358	- 218
$(ZWZBF_1xNZB)F_1$	--	10	- 10
	2,058,791	1,571,372	+ 487,419
Pregnants	143	366	- 223
Outbred Mice			
Cr:MGAPS(S)	23,148	10,132	+ 13,016
-pregnants	--	41	- 41
Cr:MGAPS(SW)	10,293	1,345	+ 8,948
	33,441	11,477	+ 21,964
Pregnants	--	41	- 41
Inbred Rats			
ACI/Cr	1,985	2,494	- 509
ACP/Cr	232	219	+ 13

TABLE I
(Continued)

Inbred Rats	1971	1970	+Increase -Decrease
ALB/Cr	414	521	- 107
AUG/Cr	1,179	978	+ 201
BN/Cr	316	315	+ 1
BUF/Cr	1,341	996	+ 345
COP/Cr	1,734	1,188	+ 546
F344/Cr	75,118	66,229	+ 8,889
-pregnants	91	3	+ 88
-litters	8	--	+ 8
LE/Cr	434	48	+ 386
M520/Cr	766	562	+ 204
NBR/PlCr	3,456	4,138	- 682
-pregnants	--	2	- 2
OM/NCr	717	17	+ 700
-pregnants	--	3	- 3
SD/MCr	2,315	1,289	+ 1,026
SH/NCr	1,184	317	+ 867
S5B/PlCr	545	736	- 191
W/FuCr	4,133	4,592	- 459
-pregnants	34	7	+ 27
W/LCr	5,348	7,457	- 2,109
-pregnants	6	19	- 13
-litters	2	2	--
YOS/Cr	1,514	225	+ 1,289
	102,731	92,321	+ 10,410
Pregnants	131	34	+ 97
Litters	10	2	+ 8
Outbred Rats			
Cr:MGAPS(Gunn)	--	40	- 40
Cr:MGAPS(OM)	34,357	1,057	+ 33,300
Cr:RAR(SD)	18,804	66,967	- 48,163
-pregnants	68	88	- 20
-litters	1	--	+ 1
	53,161	68,064	- 14,903
Pregnants	68	88	- 20
Litters	1	--	+ 1
Hamsters			
Cr:RGH(SYR)	38,573	55,811	- 17,238
Pregnants	768	117	+ 651

TABLE I
(Continued)

GRAND TOTALS	1971	1970	+Increase -Decrease
Mice	2,740,088	2,126,921	+ 613,167
Pregnants	12,288	11,065	+ 1,223
Litters	323	181	+ 142
Inbred Rats	102,731	92,321	+ 10,410
Pregnants	131	34	+ 97
Litters	10	2	+ 8
Outbred Rats	53,161	68,064	- 14,903
Pregnants	68	88	- 20
Litters	1	--	+ 1
Hamsters	38,573	55,811	- 17,238
Pregnants	768	117	+ 651
	2,934,553	2,343,117	+ 591,436
Pregnants	13,255	11,304	+ 1,951
Litters	334	183	+ 151
Dogs	564	401	+ 163
Guinea Pigs	123	207	- 84
Gerbils	83	320	- 237

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

With an accelerated national effort on the attack on cancer, an intensified expanded research and development effort in all areas will result. One major approach is via chemotherapy. A basic part of that chemotherapy is the development and utility of new and more effective drugs.

Objectives

The Drug Development Branch is engaged in a cooperative approach to the problems of cancer chemotherapy through the research and development of more effective chemical agents for use in humans. Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information is accumulating and greater achievement being experienced in the control of the more rapidly proliferating phase of cell growth, the search for new structural designs aimed against the slower growing tumors and of specific tumor areas, i.e. lung, colon, central nervous system, etc., will be increased.

As part of the broad Chemotherapy Program, the Drug Development Branch has three major objectives:

I. Fundamental Chemical Research

a. chemical synthesis of new compounds;

b. extraction, isolation and identification of new active agents from natural sources;

c. theoretical design for new chemical structures via structure-activity evaluations in collaboration with investigators of biochemistry, biology, toxicology, pharmacokinetics, and clinical trials; and

d. development of methodology for drug assay.

II. Applied Services

a. chemical resynthesis and production of active agents;

b. quality control and analytical evaluation of drugs and bulk chemicals for clinical and preclinical toxicological evaluations;

c. development and production of suitable dose formulations for use in clinical studies;

d. storage and distribution of bulk chemicals and clinical dose formulations;

e. accessioning, chemical nomenclature, development, maintenance and utilization of a computer data bank for chemical and drug information of materials entering the program; and

f. the correlation of chemical structure to biological activity.

III. Worldwide Surveillance

a. information on chemicals and drugs; and

b. sources and collections of new chemicals and drugs for biological evaluation and use in the program.

Implementation of Program

These objectives are carried out through the activities of the Branch's four Sections and its laboratory program which are:

I. Chemical and Drug Procurement Section. The objectives are divided into the synthesis, preparative, and quality control activities. The synthesis activities are involved with the synthesis of new compounds of diverse structures for antitumor evaluation.

The preparative activities include the process development and improvement of procedures for large scale production; the synthesis and isolation of large quantities of bulk chemicals and drugs for advanced screening, confirmational testing, pharmacological and toxicological evaluation, and clinical studies; and the preparation of high purity radiolabeled materials for the Chemotherapy Program. In addition, the procurement via purchase of large quantities of certain bulk chemical and drugs needed by the Program are included in the preparative activities.

II. Natural Products Section. The objective is the development of novel materials from: 1) higher plants and animal (marine and insect) sources and 2) microbial sources. In support of these tasks, the Section maintains a worldwide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, fractionation of active extracts and the isolation and identification of the active components; and in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clincal studies. Also, the Natural Products Section directs a program for the investigation of two groups of organisms, the actinomycetes and the fungi which have shown remarkable biosynthetic capabilities. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

III. Chemical and Drug Records Section. The objectives are to 1) assist in the worldwide surveillance and collection of new drugs and provide the program with possible sources of such agents, 2) manage the acquisition of chemicals contributed to the Program, 3) provide systematic names for all compounds entering the Program, 4) insure the usage of proper nomenclature for all compounds and drugs in publications emanating from the Chemotherapy Program, 5) develop, maintain, and utilize a chemical data bank on materials of use to the program, 6) develop structure-activity methodology for new materials to be synthesized in collaboration with the Chemical and Drug Procurement Section and the Laboratory Research on Chemical Drug Design, 7) maintain adequate storage and inventory of bulk chemicals, and 8) coordinate the distribution of materials to screeners and other investigators. Surveillance is also provided over industrial "Commercial Discreet" products.

IV. Clinical Drug Distribution Section. The objective is to provide the clinical program with formulated drugs in sufficient quantity and of uniform quality. Such activity includes the development of new dosage forms for clinical investigations, the surveillance of the quality of such dose forms and the maintenance of adequate supply and distribution of such drugs to the clinical investigators. In addition, the Section provides the necessary supportive data for the filing of IND applications with the Food and Drug Administration.

V. Laboratory Research on Chemical Drug Design. The objective of the laboratory effort is to provide supportive basic chemical knowledge on drug design for the Chemotherapy Program via chemical synthesis of compounds of interest, the identification and preparation of drug metabolites, the determination of physical-chemical properties of active materials and research in new methodology of drug formulation.

Even though the laboratory is only partially staffed, research in the synthetic, instrumental and drug formulation areas is underway. Current individual Progress Reports, Serial Nos. NCI-3580, 3581 and 3584 are presented later in this report.

Branch Staff

The Drug Development Branch is composed of a staff of 32 persons consisting of six senior professional scientists (GS-13 and above), eight other professional staff members (GS-9 and above), three PHS Commissioned Corps Officers, three Guest Workers, three Visiting Fellows, and the remainder technical and clerical staff.

The Branch is divided into four Sections and those responsible for each activity are as follows:

Drug Development Branch
Chief -- Dr. Harry B. Wood, Jr.

- Chemical and Drug Procurement Section
  Head -- Dr. Robert R. Engle
- Natural Products Section
  Head -- Dr. Jonathan L. Hartwell
- Clinical Drug Distribution Section
  Head -- Mr. J. Paul Davignon
- Chemical and Drug Records Section
  Head -- Mr. Robert B. Ing

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section. The Antibiotic Unit (Head, Dr. A. R. Stanley) operates as an activity under the Natural Products Section. The Chemical Laboratory (Head, Dr. John S. Driscoll) operates as an activity under the Office of the Chief of the Branch.

Operation of Program

The methods for selecting new agents for input into the primary screen is gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. Random sampling of the chemical universe cannot be abandoned completely because it continues to enjoy success in providing active materials for the program. However, as the structure-activity mosaic gradually takes shape, the choice of synthetic compounds for introduction into the screen will emerge more from direct synthesis and will gradually replace random sample screening. The gradual mutation from randomization to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

New chemical structures or analogs, developed through biochemical, cell cycle kinetics studies, pharmacological and pharmaceutical rationales and biological activities demonstrated in non-cancer programs, should provide additional input to the directed synthesis approach. Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations.

Directed synthesis will also be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

In addition to the search for "mitotically linked drugs", directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which have a high degree of organ localization should be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood brain barrier. A program has been developed to synthesize CNS active antitumor agents. Also, drugs localizing in the lung, kidney, colon, prostate, etc., will be considered in modifying their chemical structure with the objective of introducing anti-tumor properties. The increasing awareness that many drugs may possess carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic analogs of known active drugs may possess superior properties because of better transport, greater resistance to degradation or greater binding to appropriate enzyme sites, produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modification could also introduce new chromophore groups to aid in determining by analysis the drug level in the host. Analogs would be used also in mechanistic studies, and biochemical investigations which include areas of drug distribution, drug metabolites, enzymatic activity and the route and rate of excretion. Related materials provide structure-activity parameters. Last, but not least, are economical considerations of drug production via better methods of synthesis.

The Branch is keenly interested in the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives to define the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements and the fact that the physical-chemical measurements are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach

may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

The Branch is expanding its source of new materials from natural sources. Natural Products have been an invaluable source of medicinal agents and new chemical structures. Plants continue to provide many extracts with significant tumor-inhibitory activity. It is important that the compounds responsible for this activity be isolated and characterized. Some will be useful in chemotherapy, others will be useful as tools in elucidating selective growth-regulating mechanisms and as templates for syntheses of more effective agents.

Microbial products have provided many highly novel antibiotics with tumor-inhibitory activity. Investigations of animal and insect products have been initiated and there is every expectation to believe these will also provide new active agents.

At some undefined future time, the major input will be synthetic compounds specifically designed for anticancer effects and prepared under contract. There will be more analogs of existing active materials, fewer new or unique structures, fewer biologic tests required, and, logically, the number of active agents should be higher and acquiring the agents will be more costly. If one were to obtain a constant number of active materials, random screening would require a much larger input of compounds than a rational approach. Unfortunately, the state-of-the-art has not reached a degree of sophistication which allows a completely rational approach to all drug design. Currently (1971), we still acquire the largest number of compounds from random sources, but an increasing number of rationally designed compounds are being generated. While the rational method is preferred, the random approach will serve a useful purpose for the foreseeable future. Random input has thus far provided the greatest number of active compounds and certainly the greatest number of unique structures.

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of "Commercial Discreet" data must be ever present. A total of 2252 individual suppliers, of which 1055 are industrial suppliers, have contributed samples to the Program. During 1971, there have been 165 new non-industrial suppliers and 42 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers, many materials have been supplied from foreign countries. The increasing number of contributors is an index of the enthusiasm for the Program by the scientific community.

The Drug Development Branch has maintained a steady flow of materials into the Program. These materials consist of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point II). A number of Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (See Table I).

In comparing 1971 with 1970, the data in Table I reflects a significant decrease in the number of crude plant extracts entering the Program. The number of crude extracts in the fermentation product category has shown a decrease while there has been an increase in the number of animal products. There has been a major increase in the number of synthetic materials entering the Program. Since the greatest number of active materials in the past has come from the synthetic category, it is expected that as the input in the Program increases, the total number of active materials will also increase. This has been the case as shown in Table I. There were 211 confirmed activities in 1971, an increase of 76 in comparison with 1970. Of these 211 confirmed actives, 195 were synthetic, 9 plant products and 7 fermentation products. During 1971, there was an increase of 10 agents over 1970 passing Decision Point II (approved for toxicology and as candidates for clinical trial). Of the 19 new Decison Point II compounds, 12 were synthetic, 3 were plant products and 4 were fermentation products. There were eight IND drugs filed for clinical use with the FDA. Of the eight there were 6 synthetic and 2 were fermentation products. There is every reason to believe these numbers will increase significantly during 1972.

Of the 10,151 new and crystalline materials added in 1971 to the Program, 881 compounds (8.7%) were acquired under contract, 169 compounds (1.7%) were purchased, 4034 compounds (39.7%) were voluntarily submitted from industrial sources and 5067 compounds (49.9%) were from non-industrial suppliers. Of the total, 3460 new materials were acquired by field collection trips.

Of the 3015 plant extracts tested, approximately 56% were from contractors, and 79 crude extracts were active. Eighty-five new crystalline materials were isolated from plant extracts and 9 have shown confirmed activity (Step III). During this period 3 materials of plant origin was selected for preclinical toxicological evaluation (Table I) and no IND's were filed (Table I).

From the 1215 fermentation extracts, 191 demonstrated activity. Twenty-six were isolated in crystalline form and 7 have shown confirmed activity (Step III). Four fermentation products have been selected for preclinical toxicological evaluation (Decision Point II) (Table I), and 2 IND's were filed (Table I).

Of the 1120 crude animal products, none have shown activity. No crystalline materials have been isolated during this period, none has shown confirmed activity (Step III) and none were approved for Decision Point II or filed for an IND.

The area of development and production of dosage forms for clinical use has continued to operate at a high level of effort. Production of parenteral products by formulation contractors increased by approximately 51% over 1970. In 1971, 14 projects for formulation development were assigned to pharmaceutical contractors. Many of these projects have been completed and the resultant experimental dosage forms have been submitted for further preclinical testing. Seven of these dosage forms were cleared for clinical distribution. Additionally, modifications requiring IND supplements were made on 15 existing formulations.

The production record achieved by formulation contractors accounts for 156,585 compressed tablets; 537,575 hard gelatin capsules and 306,506 vials. A total of 642,021 drug items (vials and bottles) were received in 1971 and 560,265 items were distributed to clinical investigators throughout the world during that same period. An average inventory of 109 different compounds involving approximately 186 different formulations has been maintained.

In certain situations it is desirable to evaluate more than one drug or a drug and placebo under the same conditions. This is done by "double-blind" clinical studies which require that the materials used are to be formulated in dosage forms that cannot be differentiated from each other except by code. There are 19 coded studies on inventory.

The Branch upon request, has reviewed and commented upon 134 Grant Proposals. The opinions expressed have been used extensively by the Grant Study Sections on their evaluation of the Proposals. Such comments may include the number of materials submitted for testing by the grantee, indicating those compounds showing promise as potential antitumor agents.

Also upon request, the Branch has reviewed 6 patent applications. The applications are reviewed as to novelty, expected utility and need to protect the public's interest by patent. The opinions and recommendations are used by the NIH Patent Section in their consideration of the application.

The Branch sponsored six scientific seminars, including leaders in chemistry and biochemistry. The second major workshop in nucleosides was held. The primary purpose of this workshop was to provide interplay between the most recent advances in biochemistry, chemistry and pharmacology in the nucleoside-nucleotide area and from such interplay of ideas and discussion new compounds in this area will be synthesized for the chemotherapy program. A similar workshop in the folic acid antagonists area is planned in the near future as well as one in the methodology of structure-activity techniques.

A number of Ad Hoc committees of top medicinal and organic chemists, and biochemists are under consideration to review various segments of the Branch's new approaches, as well as new areas not yet explored will be considered.

The Branch's laboratory operation has made significant technical progress on projects related to the synthesis of azapurines and triazole agents potentially active against brain tumors. Analytical research in high resolution NMR spectroscopy has also been investigated. A total of 48 compounds

were submitted by the Drug Development laboratories for biological evaluation. In addition, studies on the decomposition of NSC-50741 [β-D-ribofuranoside, methyl-5-(2-chloroethylamino)-5-deoxy-2,3-O-isopropylidene-, hydrochloride] have been pursued and fractions with T/C greater than 200 in L-1210 have been found.

The Branch manages a total of 49 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately six million dollars and covering the major areas as shown under the primary responsibilities of the Branch. Of the 49 contracts, 46 are located in the continental United States and three are foreign contracts.

Summary of Branch Achievements

In summary, the following were achieved during the calendar year 1971 by the Drug Development Branch:

1. A total of 15584 new substances (Table I) was obtained for screening or other evaluation;

   a. 10040 synthetics

   b. 1215 antibiotic cultures

   c. 3015 plant materials

   d. 1203 animal materials

   e. 111 other crystalline materials

2. A total of 984 compounds were prepared under contract:

   a. 103 were resynthesized by the preparation laboratory contracts

   b. 858 were synthesized as new compounds from synthesis and prep lab contracts

   c. 23 were from the natural product laboratory contracts

3. Nineteen new substances were assigned to Decision Point II (Table I and II). (Candidates for preclinical toxicology);

   a. 4 fermentation products

   b. 3 plant products

   c. 12 synthetic compounds

4. Eight drugs were assigned IND numbers (Table I and III).

   a. 2 fermentation products

   b. 6 synthetic compounds

5. Two hundred eleven compounds were assigned to Step III (Table I).

6. Analytical-quality control determinations were made on 160 different lots of 71 different compounds representing 83 lots of bulk compounds and 77 lots of clinical formulations.

7. Sixteen radiolabeled compounds (Table V) were procured.

8. Fourteen new dosage forms were developed and fifteen existing dosage forms were modified.

9. Approximately 3,415,392 formulated units were procured.

   a. 2,203,905 compressed tablets

   b. 602,175 hard gelatin capsules

   c. 609,312 vials

10. Forty-nine contracts (some multi-phase contracts) were supervised by the Branch;

    a. 10 preparation laboratories

    b. 15 new synthetics

    c. 10 plant products

    d. 4 fermentation products

    e. 1 analytical-quality control

    f. 7 drug formulations

    g. 2 miscellaneous

11. There were 134 Grant Proposals and 6 Patent Applications reviewed and commented upon.

12. Samples were received from 42 new industrial concerns.

13. Materials were received from 165 non-industrial sources.

14. Sixty-two manuscrips by contractors were cleared for publication.

15. Twelve manuscripts were published by the Branch staff.

16. Six scientific seminars were sponsored.

17. One workshop on nucleosides was sponsored.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT (1)

	CRUDE EXTRACTS NO. SUBMITTED		CRUDE EXTRACTS NO. ACTIVE		CRYSTALLINE MATERIAL SUBMITTED		STEP III (2)		DECISION POINT II (3)		IND FILED	
	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971
Fermentation Products	2172	1215	104	191	48	26	7	7	3	4	2	2
Animal Products	587	1203	22	-	-	-	1	-	-	-	-	-
Plant Products	3987	3015 (4)	119	79 (4)	64	85	12	9	1	3	2	-
Synthetics	--	--	--	--	8409	10040	115	195	3	12	3	6
TOTAL	6746	5433	245	270	8521	10151	135	211	7	19	7	8

(1) Based on calendar year 1971.

(2) Showing confirmed activity.

(3) Selected as candidates for preclinical toxicological evaluation and clinical trial.

(4) Individual species.

TABLE II

COMPOUNDS PASSING DECISION POINT II DURING CALENDAR YEAR 1971

NSC NO.	NAME	SOURCE
12,169	Estriol	Synthetic
74,437	Piperazine, 1,4-bis(2,3-epoxy-propyl	Synthetic
96,349	Coralyne	Plant Product
126,849	3-Deazauridine	Synthetic
129,220	Cyclocytidine, acetate	Synthetic
145,668	Cyclocytidine, HCl	Synthetic
132,313	Galactitol, 1,2:5,6-dianhydro-	Synthetic
133,100	Rifamycin SV	Fermentation Product
135,962	Palmo-Ara C	Synthetic
139,105	Ethanesulfonic acid, compd. with α-[2-chloro-4,6-diamino-2,2-dimethyl-s-triazin-1 (2H-yl)phen-oxy]-N,N-dimethyl-m-toluamide (1:1)	Synthetic
141,537	Anguidine	Fermentation Product
141,540	Epipodophyllotoxin,4'-dimethyl-, 4,6-O-ethylidene-β-D-gluco-pyranoside	Synthetic
141,549	Methanesulfon-m-anisidine,4'-(9-acridinylamine)-, monohydro-chloride	Synthetic
143,647	DL-Alanosine	Fermentation Product
143,769	Methanol extracted residue of BCG (MER)	Fermentation Product
143,969	Tilorone	Synthetic
145,669	4(1H)-Quinazolinone, 2,3-dihydro	Synthetic
146,396	Nitidine, 12,13-dihydro-13-methoxy	Plant Product
146,397	Nitidine chloride, dihydrate	Plant Product

TABLE III

NEW IND APPLICATIONS FILED in 1971

NSC NO.	NAME	IND NO.
95,441	Methyl-CCNU	7681
102,627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester) hydrochloride	7575
102,816	5-Azacytidine	7574
109,724	Ifosfamide	7887
119,875	Platinum, diamminedichloro-, cis-	8041
129,943	ICRF-159	7651
133,100	Rifamycin SV	8262
406,021	9H-Purine-6-thiol, 9-β-D-arabinofuranosyl	7576

Serial No. NCI 3580
1. Chemotherapy, DR&D
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chemical Research in the Development of New Anticancer Drugs

Previous Serial Number: NCI 3580

Principal Investigator: John S. Driscoll

Other Investigators: Tadashi Hirata, Ashwani Malhotra, A. Hameed Khan, Victor Marquez, Robert Engle, Harry B. Wood, Jr.

Man-Years

Total: 3.4
Professional: 2.8

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. This goal is pursued using leads developed through structure-activity correlations, the data from the random screening of compounds, and literature biomechanistic rationale.

Candidate drugs can originate either as synthetic materials or may be isolated from active natural product extracts. Natural products with proven effectiveness are also synthesized.

Specific Objectives:

1. Studies of the L1210 active decomposition products of NSC-50741.

   a. The preparation of active mixtures; the isolation and identification of components.

   b. Synthesis and decomposition of analogs of NSC-50741 to determine critical structural parameters.

2. The synthesis of CNS active antitumor drugs.

3. The synthesis of bridge-head nitrogen heterocyclic compounds.

Major Findings:

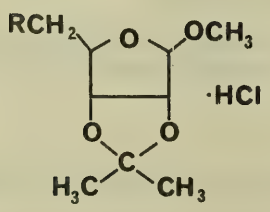
1. The Active Decomposition Products from NSC-50741

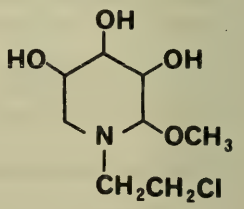
a. Preparation and fractionation of L1210 active mixtures (A. Malhotra)

Pure NSC-50741 (1a) is inactive in the L1210 test system. It was found that upon standing for 5 years at 4°, NSC-50741 decomposed to an L1210 active mixture. The objective of this project is the acceleration of the production of the active mixture, as well as the isolation, identification, and synthesis of the active component.

Reproducible production of an active mixture was achieved. Many different variables were found to influence the activity of the product. The temperature of the incubation solution was especially important. Also critical were the lyophilization procedure and the temperature at which the solid was stored. After standardization of the preparation method, the incubation mixtures were found to be L1210 active on all three treatment schedules used. While some irreproducibility among duplicate samples has been observed, T/C values of 175% are normal, while values of >225% are no longer unusual.

Fractionation of the mixture into its component parts has proven to be very difficult. Dry column chromatography at 4° appears to be most suitable. Thin-layer chromatography indicated four major components in the active mixture. Two of these have been isolated and identified. Biological testing has shown them to be inactive. The yields of the other two fractions are low and the materials appear to be thermally unstable. Preliminary structural information indicate the possibility of structures similar to (2) for the presently unknown components.





1a, R = $NHCH_2CH_2Cl$

1b, R = $NHCH_2CH_2CH_3$

1c, R = $NHCH_2CH_2OC_2H_5$

1d, R = $N(CH_2CH_2OH)(CH_2CH_3)$

1e, R = $N(CH_2CH_2OH)_2$

1f, R = $N(CH_2CH_2Cl)(CH_2CH_3)$

1g, R = $N(CH_2CH_2Cl)_2$

1h, R = NH_2

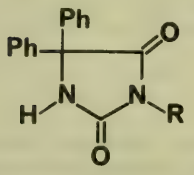
2

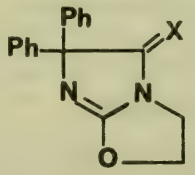
b. Synthesis and thermal reactions of analogs of NSC-50741 (A. H. Khan)

The search for new antitumor agents based on the active decomposition product of NSC-50741 (1a) is being pursued by a second approach. If the critical structural parameters relating to the conversion of 1a into an active compound were known, (a) insight into the structure of the active material would be gained, (b) the relatively tedious and inefficient isolation procedure might be bypassed, and (c) the chances of direct synthesis of the active component would be greatly enhanced. A project in this area was initiated in late 1971. The objective is to synthesize several selected analogs (1b-h) of NSC-50741 in order to test both pure and decomposed samples in the L1210 test system and compare the results with those obtained with active decomposed samples of NSC-50741. Major questions to be answered are (a) is the β-chloroethyl group of 1a necessary for activity, (b) is a secondary amine required, and (c) are there other derivatives capable of yielding even higher activities upon decomposition? Compound 1b and 1c have been prepared in order to answer (a) above. Compounds 1d and 1e have been synthesized as intermediates to 1f and 1g in order to answer question (b). These materials, with the exception of 1f, 1g, and 1h have been prepared and characterized. Sufficient quantities are currently being synthesized to allow the decomposition reaction to be studied. A number of other analogs are also being prepared.

2. The synthesis of CNS active antitumor drugs (V. E. Marquez)

Diphenylhydantoin (DPH) derivatives with alkylating properties were considered as potential agents for the treatment of CNS and brain tumors. Approximately 15 members of this family were prepared and tested in the L1210 system. While some were quite toxic, none were active. Typical structures synthesized are shown in (3) and (4).





3a, R = $CH_2CH(O)CH_2$

3b, R = $CH_2CH_2N(CH_2CH_2Cl)_2$

4a, X = O

4b, X = H_2

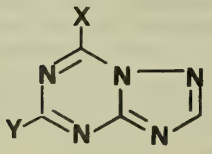
Most of these compounds were very strong alkylating agents by the chemical NBP test. It is felt that the lack of antitumor activity may be due to too large an increase in the lipophilic character of the derivatives, relative to DPH. This project has been temporarily suspended since October 1971.

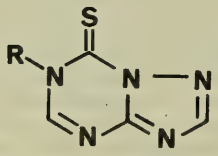
Several good leads for compounds with L1210 activity which might also have CNS antitumor activity were uncovered during the selective rescreening of derivatives with known psycotropic activity. Five L1210 active phenothiazines have been found among the 16 tested. We hope to exploit these leads synthetically during 1972. In addition, we plan to greatly increase the routine screening of known psycotropic agents (tranquilizers, etc.) in order to generate further leads in this area.

3. Bridge-head Nitrogen Heterocyclic Compounds and Their Intermediates (T. Hirata)

a. 5-Azapurines

The 5-azapurine system (5) has been little explored either chemically





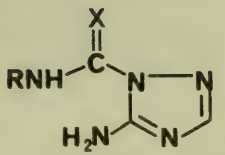
5a, X = SH; Y = OH
5b, X = OH; Y = SH
5c, X = OH, Y = OH
5d, X = OH, Y = H

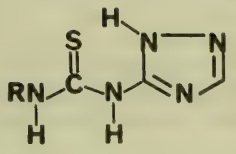
5e, R = CH_3
5f, R = CH_2Ph

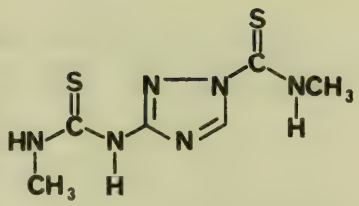
or chemotherapeutically. Six compounds of this type (5a-f) were prepared and screened. None proved to have L1210 activity. This project is, therefore, being terminated and effort is being shifted to more promising families of compounds.

b. 1,2,4-Triazoles

An extensive study was carried out on isomeric products obtained when 3-amino-1,2,4-triazole was reacted with isocyanates and isothiocyanates. Approximately a dozen new derivatives were prepared. Typical of these new compounds are (6-8). The purpose of the study was (a) to provide the required intermediates for the 5-azapurine family, (b) to prepare and test derivatives related to desamino-guanazole, and (c) to clear up the confusion in the literature relative to the actual isomers produced among the four that are possible. All of these goals were accomplished. It was found that certain of these derivatives very easily underwent thermal rearrangement from compound type 6 to 7. Also, base catalyzed elimination of HNCS in compounds such a 6c was discovered to occur. These previously unrecognized reactions are probably responsible for much of the conflicting literature data. The new triazoles were screened and found to be inactive. This investigati has, therefore, been terminated.







6a, X = S;R = CH_3 or $PhCH_2$

6b, X = O;R = CH_3 or CH_2CH_2Cl

6c, X = S;R = C_2H_5CO

7a, X = S; R = $NHCH_3$

7b, X = O; R = C_2H_5

8

c. Methylation of 5-Azapurines

As a separate part of the investigation of bridge-head nitrogen compounds, the reaction of four 5-azapurine derivatives with various types of alkylating (methylating) agents was studied. The isomeric materials produced under various conditions are being structurally identified by high resolution nmr spectroscopy and mass spectrometry. Because of the previously mentioned lack of L1210 activity among the 5-azapurines synthesized thus far, this study currently has low priority and will soon be completed.

PUBLICATIONS

Hirata, T., Twanmoh, L-M., Wood, H. B., Goldin, A., and Driscoll, J. S.: 5-Azapurines and the Structures of sym-Triazole Intermediates. J. Heterocyclic Chem. 9: 99-106, 1972.

Marquez, V. E., Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: Intramolecular Cyclizations Leading to N-Bridgehead Bicyclics. J. Organic Chem., in press.

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Paull, K. D., Engle, R. R., Twanmoh, L-M., Wood, H. B., and Driscoll, J. S.: The Synthesis of 9-(3,4-Dimethoxyphenoxy)-1,2,10-trimethoxyaporphine. J. Pharmaceutical Sci., in press.

Serial No. NCI 3581
1. Chemotherapy, DR&D
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Analytical Chemistry of New Anticancer Drugs

Previous Serial Number: NCI 3581

Principal Investigator: John S. Driscoll

Other Investigators: Li-Ming Twanmoh, Robert R. Engle and Harry B. Wood, Jr.

Man Years

Total: 1.2
Professional: 1.2

Project Description:

General Objectives:

The object of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;

2. establish the identity and purity of bulk and formulated drugs;

3. determine important physical and chemical properties of new anticancer drugs;

4. study reaction mechanisms and structure-activity relationships.

Method Employed:

Emphasis is placed on modern instrumental techniques. Primary emphasis is placed on the use of high resolution nmr spectroscopy as an analytical tool. Chromatographic techniques, as well as infrared and ultraviolet spectroscopy, are also extensively employed.

Major Findings:

1. Structural Studies (L-M. Twanmoh)

a. Azapurines

The structure of a number of new 5-azapurine derivatives and their intermediate reaction products were investigated and confirmed by nmr spectroscopy. The spectral properties of this relatively unexplored heterocyclic system have been cataloged.

b. Potential CNS Antitumor Agents

The structures of 12 new diphenylhydantoin derivatives, designed and synthesized as CNS antitumor agents were confirmed. The direction of a unique intramolecular cyclization reaction was determined using nmr techniques. An unusual room temperature methylene-hydroxyl coupling was detected.

c. Aminosugars

A number of decomposed NSC-50741 fractions were examined by nmr spectroscopy in an attempt to determine the structures of the components. This has not yet been successful because pure samples of the unstable material have not been isolated.

Several new pure aminoribose derivatives, synthesized as NSC-50741 analogs, were characterized.

d. Miscellaneous NMR Spectral Studies

(1) NIAMD

Seven structures were determined for the Laboratory of Chemistry.

(2) USDA

Five samples were run for Dr. Dutkey of the Insect Pathology Group.

(3) Microbiological Associates

Eighteen samples were determined for the chemistry group of Microbiological Associates.

(4) Harry Diamond Laboratory

The 100MHz coupling constants of several compounds were determined for Dr. Lee.

2. Mechanistic Studies (L-M. Twanmoh)

a. Purine N-H Protons

An observation that N-H protons in purine and azapurine derivatives were very difficult to detect, coupled with an almost total lack of literature data, lead to a study of the characteristic chemical shifts

of protons attached to ring nitrogen atoms in appropriate derivatives. Standard purines such as hypoxanthine and xanthine, as well as new azapurines, were characterized and the absorptions were assigned. Traces of the water (<0.1%) in the non-aqueous solvents used were determined to have a greater effect on the ability to observe an N-H signal than the nitrogen quadropolar effect.

b. Triazole Rearrangement Reactions

The kinetics of the thermal rearrangement of thiouredo derivatives of sym-triazole were determined by nmr spectroscopy. The rate of disappearance of the starting materials as well as the rate of build-up of intermediates and products was easily recognizable.

3. Assay and Physical Property Measurement (L-M. Twanmoh and J. Driscoll)

a. Methotrexate Derivatives

The use of Fourier transform (F-T) nmr spectroscopy as an analytical tool was studied using folic acid and MTX derivatives as model compounds. The purpose of the study was to determine the lowest concentration of drug that would give a usable spectrum. While the normal concentrations used in nmr spectroscopy approximate 5 percent, it was found that a useful spectrum of the disodium salt of folic acid in D_2O could be obtained on a 8.5×10^{-4} percent solution after the accumulation of 2500 transients. The general applicability of this technique to the microanalytical chemistry of drugs of interest to the program is being explored further.

The nmr spectra of 10 methotrexate derivatives were determined and analyzed. As soon as appropriate biological test data are available, structure-activity correlations will be attempted using the chemical shift of the N-methyl groups as the structural parameter.

b. Triazine Antifol (NSC-139105)

Different observations by various workers as to the solubility of this material lead to nmr and thermal studies of the material. Preliminary data indicate that NSC-139105 exists as a hydrate in aqueous solution, and that the hydrate may be more soluble than the unhydrated material.

c. Partition Coefficients

The octanol-water partition coefficient of methylnitrosourea was determined to be -0.160 ± 0.013 by ultraviolet spectroscopy. This value was used in the calculation of a π value for the nitrosourea group.

d. Melphalan (NSC-8806)

The purity and identity of a sample of melphalan and one of its derivatives was determined for the Drug Evaluation Branch by nmr analysis.

CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to synthesize new compounds for antitumor evaluation, to procure large quantities for advanced screening, confirmational testing, toxicological and pharmacological evaluation, and clinical trial in humans, and to procure radiolabeled materials needed for pharmacological and clinical studies.

The work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;

2. The Preparative Activities, which synthesize

   a. bulk chemicals and drugs

   b. radiolabeled materials

   for toxicological, pharmacological and clinical investigations, for confirmational testing and for completion of primary evaluation; and

3. Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the pharmacological and clinical programs. Solubility and stability data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section is presently composed of three professional people and one secretary. One professional person is involved full time with the quality control activities of the Section. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;

2. Leads generating from the screening program;

3. Biological and biochemical rationale;

4. Leads from the literature;

5. Compounds to explore structure-activity relationships;

6. New structural types; and

7. Agents to resolve questions on chemical and biochemical mechanism and mode of action.

During the Second Joint Working Conference, National Cancer Institute Chemotherapy Program, a Workshop was held on Selection of Compounds for Synthesis. As noted above, different criteria are used to select compounds for synthesis. During the workshop the following methods for selecting compounds to synthesize were discussed:

1. Biologic Rationale

2. N-O-O-Triangulation

3. Intercalation

4. Structure-Activity Correlation

5. Screening Results

6. New Structural Classes

It was concluded that no one method is best and that all methods discussed, as well as others not discussed at this meeting, are useful in the selection of compounds for synthesis.

The efforts of the synthetic program are channeled into many areas of investigation. The following are some examples:

(1) A significant amount of the synthetic effort of the Section has been directed towards the synthesis of nucleosides. The area of nucleosides has already provided a number of very interesting compounds capable of suppressing tumor growth in experimental animals and producing favorable clinical response in certain types of human cancer. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to undesirable enzymatic degradation in the cancer patient.

In addition, the study of nucleoside metabolism may be expected to contribute to the design of chemotherapeutic agents with antiviral activity against tumor-inducing viruses and may help elucidate many cellular processes in the synthesis of DNA and therefore in cell division. Nucleoside antimetabolites may function by inhibiting the incorporation of normal constituents in RNA or DNA and preventing the formation of enzymes necessary for biosynthesis of the tumor nucleic acids. Also, the conversion of nucleosides to nucleotides may be structurally less restrictive than

the conversion of the free base to the corresponding nucleotides.

A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute, Stanford Research Institute and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) pyrrolo[2,3-d]pyrimidine nucleosides (c) tricyclic nucleosides (d) nucleosides of pyrazolo[3,4-d]pyrimidine, pyrazolo[4,3-d] pyrimidine, imidazo[4,5-c]pyridine and imidazo[4,5-b]pyrazine (e) indazole, imidazole and benzimidazole nucleosides (f) 3',5'-cyclic monophosphate derivatives (g) uronic acid nucleosides (h) "carbocyclic sugar" nucleoside derivatives (i) "carbon-carbon nucleosides" (j) "isonucleosides" (base attached at C-2 of the sugar moiety), (k) fluorinated aminonucleosides, (l) deazapurine nucleosides and (m) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars.

During this report period a Nucleoside Workshop was held. This was the second such conference to review and discuss this interesting area of chemistry by the Drug Development Branch. The purpose of the Workshop was to give guidance as to whether or not this was still a fruitful area of investigation and if so, where the effort should be directed, and to provide for an interplay of ideas and discussion between the chemist, biochemist and pharmacologist working in the area. It was concluded that the nucleoside area has been very fruitful and several compounds of clinical interest belong to this class of compounds. Also, that there are some materials of this type which are showing interesting activity in preclinical evaluation. It was also concluded that there is an abundance of work yet to be done in this area of chemistry. Some areas need to be investigated further, especially the 2'-deoxy nucleosides, C-nucleosides and fraudulent sugar nucleosides, while in others the investigations have only recently been initiated or yet to be initiated.

It was the consensus of opinion of those attending the workshop that it was a very useful conference and more such meetings should be held. Additional workshops in the area of nucleosides are being planned as well as in other areas of chemistry.

(2) A second major effort of the synthesis program has been the preparation of analogs and derivatives of folic acid. Resistance to folic acid antagonists in L-1210 leukemia is frequently associated with a marked increase in the enzyme dihydrofolate reductase. Since this change is limited to tumor cells, the provision of a fraudulent substrate to the tumor cell would lead to the production of a fraudulent coenzyme, thus producing a lethal synthesis in the tumor cell. Homofolic acid was prepared as a potential fraudulent substrate for dihydrofolate reductase in resistant tumor cells. Tetrahydrohomofolic acid, the end product of the reduction of homofolic acid by dihydrofolate reductase, has also been prepared and is a striking inhibitor of thymidylate synthetase.

Tetrahydrohomofolic acid was selected as a potential clinical candidate, however, its poor stability proved to be a serious drawback. The 5-methyl derivative of tetrahydrohomofolic acid has been shown to have better stability properties and still retains the antitumor activity. 5-Methyl-tetrahydrohomofolic acid is presently being obtained in large quantities for further development.

New folic acid analogs and derivatives have been prepared by Southern and Stanford Research Institutes. The structure of the folic acid molecule has been divided into five parts, (a) Ring A (pyrimidine) of the pteridine ring, (b) Ring B of the pteridine ring, (c) the Bridge, (d) the Benzene portion (Ring C), and (e) the Amino Acid moiety. Modifications have been undertaken in each area.

(3) A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

A practical total synthesis of the naturally occurring alkaloid coralyne was achieved during this report period by Midwest Research Institute. Large quantities of this alkaloid are needed for toxicologic and clinical studies and to obtain the amounts needed from natural sources would be both difficult and expensive. Large quantities of the alkaloid are currently being prepared via the synthetic route.

Work on the preparation of analogs and derivatives of the clinically effective antibiotic daunomycin and adriamycin has continued at Stanford Research Institute. This work has been directed in the following two main areas: (1) The preparation of a series of amino and carbonyl derivatives of the parent antibiotic since recent work has indicated that such derivatives may display reduced toxicity while retaining antitumor properties; and (2) The preparation of more accessible synthetic analogs, mainly substituted naphthacenequinones and anthraquinones, with basic side chains that stimulate the aminosugar portion of the parent molecule. Work in this area in continuing.

Work has continued on the preparation of analogs and derivatives of the active alkaloid ellipticine. The main effort during this report period has been devoted to the preparation of close relatives bearing various combinations of substituents on the 1, 5, and 6 positions. Work in the ellipticine area was done at Stanford Research Institute and Central Drug

Research Institute (India) and is continuing.

Another alkaloid on which some synthetic effort has been extended is thalicarpine. The work towards the total synthesis of the parent compound was discontinued during the report period when a synthesis of the compound was reported in the literature. However, work on the preparation of some analogs and derivatives has continued. This effort has been directed mainly towards the preparation of the nitrogen isosteres of thalicarpine, hernandaline and related methylenedeoxy derivatives.

A limited amount of synthetic effort during this report period was directed towards the total synthesis of the naturally occurring alkaloid camptothecin, and the preparation of simplified synthetic analogs of the parent compound. The major portion of this work has been discontinued due to the reduced clinical interest in the parent compound.

Some of the synthetic effort of the Section has been devoted to the preparation of some structural moieties of the antitumor antibiotics, Bleomycin. Bleomycin is a mixture of closely related antibiotics, the chemical structures of which have not been completely elucidated. It is known that bleomycins contain polypeptides composed of a substituted bithiazole among others. Several bithiazoles and related thiazoles have been prepared by Midwest Research Institute.

(4) Some of the effort of the Section has been directed towards the synthesis of other potential antimetabolites. One such structural type is the imidazole. Two imidazole-containing compounds have demonstrated activity against L-1210 leukemia and are undergoing clinical evaluation. In addition, some compounds of this type have been shown to have broad spectrum antifungal and antibacterial activity. Thus, additional imidazole analogs and derivatives have been prepared and are being synthesized for evaluation as antitumor agents.

(5) The class of compounds known as N-nitrosoureas are active against leukemia and some possess the ability to cross the blood-brain barrier. Of special interest are the results from intracerebrally-inoculated L-1210 leukemia, Lewis Lung carcinoma, and the use of maximal single dose (one day only) treatment schedules. The testing in rodents with some compounds of this type has resulted in a significant number of cures based on thirty-day survivals. Additional examples of this structural type have been synthesized by Southern Research Institute and submitted for antitumor evaluation.

(6) A limited amount of effort has been devoted to the synthesis of analogs of guanazole, which has demonstrated activity against L-1210 leukemia and is currently undergoing clinical evaluation. In addition, structural modifications of the *in vivo* antileukemic active nicotinohydroxamic acid has been undertaken. Interest in the hydroxamic acid type of compound has been enhanced by the recent observation that some compounds of this type are also inhibitors of ribonucleoside diphosphate reductase. Several compounds of this class have been prepared.

(7) A limited amount of effort has been devoted to the isolation and synthesis of metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation *in vivo*. Work in this area is continuing in an effort to determine the mechanism of activation of this active agent and to isolate and synthesize the active metabolite(s).

(8) Some of the synthetic effort of the Section has been directed towards the synthesis of compounds which potentially may localize in specific organs or parts of the body. The main effort in this area during this report period has been to develop CNS active antitumor agents. Some derivatives of ergocornine, a CNS active natural product in the lysergic acid family, have been prepared. In addition some heterocyclic azepines and some acetylenic oxazolones and their ethynyl carbamate precursors have been prepared. The work in this area was done at the Sheehan Institute for Research and at the University of Georgia.

(9) The synthesis of some new nitrogen heterocycles as potential antitumor agents has also been undertaken during this report period. The emphasis in this area has been on the preparation of bridgehead nitrogen heterocycles, especially hydropyrimidine derivatives and pyridazine compounds; imidazo[1,5-a]pyrazines, isoxazolo[4,5-b]pyrazines and their analogs; and pyrazolotriazines, pyrazolopyrimidines, pyrazolopyrazines, pyrazoloimidazopyrazines, imidazopyrazines, pyrrolopurines and indenopyrimidines. Several compounds of these types have been prepared and submitted for testing. The contractors at the University of Michigan, University of Rhode Island and Dow Chemical Co. have been working in this area.

(10) In addition to the effort devoted to the synthesis of nitrogen heterocycles as new structural moieties as potential antitumor agents, a limited amount of synthetic effort was devoted to the preparation of new sulfur/oxygen heterocycles. The emphasis in this endeavor has been to prepare some 1,2,4-trithiolanes, cyclic disulfides, dithietanes and related compounds. Several compounds of these types were prepared by Ash Stevens, Inc.

(11) A few oligo and polynucleotides were prepared as potential inhibitors of the RNA dependent DNA polymerases and other DNA polymerases. This work was done at Collaborative Research, Incorporated.

(12) In order to develop the discovery of the high degree of antitumor activity in the coordination compound *cis*-diamminedichloroplatinum which is currently being studied clinically, a contract has been initiated with Pennwalt Corporation to prepare complexes of some of the transition metals. During this report period several complexes of platinum, rhodium, niobium and tantalum have been prepared. Work in this area is continuing and is being expanded to include the coordination compounds of cobalt, iron, nickel, gallium, indium and thallium as well as additional examples of the four metals noted above.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Chemotherapy Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure.

During the past year, 19 new compounds passed Decision Point II, of which 12 were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs 16 contracts for the chemical synthesis of new compounds for the Program (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR SYNTHESIS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ash-Stevens, Inc.	Ash	NIH-71-2314
British Columbia, University of	Kutney	NIH-72-3223
Central Drug Research Institute	Dhar	01-004-1 (PL 480)
Collaborative Research, Inc.	Friedman	NIH-71-2326
Dow Chemical Company	Collins	NIH-71-2313
Georgia Institute of Technology	Zalkow/Gabriel	PH-43-68-1288
Georgia, University of	Blanton/Martinelli	NIH-71-2311
Michigan, University of	Burckhalter	NIH-69-2004
Microbiological Associates, Inc.	Beisler	PH43-68-1283
Midwest Research Institute	Cheng	PH43-65-94
Pennwalt Corporation	Block	NIH-71-2309
Rhode Island, University of	Goodman/Abushanab	NIH-71-2312
Sheehan Institute for Research	Razdan	NIH-71-2207
Southern Research Institute	Montgomery	NIH-71-2021
Stanford Research Institute	Henry	NIH-71-2070
Utah, University of	Townsend	PH43-65-1041

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not available from the original supplier or on the open market. These laboratories differ from the synthesis contract laboratories in that the synthesis contracts are devoted entirely to the preparation of new compounds with diverse chemical structures for primary screening. The preparative laboratories also perform the large scale synthesis, extraction, and isolation of materials from natural sources. The laboratories were selected on the basis of ability of the principal investigator, the facilities available, the capability of the company in the field of chemistry related to the Chemotherapy Program and the adaptability to synthesis of all types of compounds.

The preparation laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. Usually methods and/or references for the synthesis of the compounds requested are supplied to the contractors by the Section. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies have also been performed by the preparation laboratories upon request. In addition, some of these contractors have provided material in formulated dose form.

The increased emphasis being extended toward the conquest of cancer has caused a major increase of the preparation activities. An increase in the number of compounds demonstrating confirmed activity will result in an increase in the number of compounds to be resynthesized for completion of screening or confirmational testing. Also, the increased effort being devoted to toxicologic and clinical studies has resulted in the need for more compounds at this level and in larger quantities. This increased effort has also resulted in the need for additional radiolabeled materials. It is anticipated that this increase in preparation activities will continue during the coming year as the Chemotherapy Program expands.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs which may be needed for the Program.

(1) Bulk chemical and drugs

The compounds chose for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons:

(a) For clinical investigations;

(b) For preclinical toxicological and pharmacological evaluation;

(c) For maintaining a stock of chemical of clinical and preclinical interest for study in the Program;

(d) For completion of screening or confirmational testing; and

(e) For use as intermediates in further synthesis.

The compounds so chosen are placed on priority lists based upon their relative importance, with the toxicological and clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to as much as 200 kilograms. Factors governing the amounts depend upon such considerations as: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 103 compounds totaling 450 kilograms during the calendar year 1971. Included in this group were 22 compounds scheduled for or undergoing clinical evaluation totaling 418 kilograms. In addition to the compounds resynthesized, 84 new compounds were also prepared by the preparative laboratories. During this report period the Section also purchased 8 compounds scheduled for or undergoing clinical evaluation totaling 93 kilograms.

(2) Radiolabeled Materials

The continued importance being placed on pharmacological and drug distribution studies has maintained the need for radiolabeled chemicals and drugs. These agents are valuable tools in such studies and the Section coordinates their procurement and distribution.

Two contracts (PH43-65-1044 and NIH-71-2162) are devoted to the procurement of radiolabeled materials for use in preclinical pharmacological and clinical studies. If available, the required labeled materials are purchased from commercial sources. Materials not available from commercial sources are prepared by these preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. During this report period, the Section procured from all sources a total of 16 radiolabeled materials (Table V) and directed their distribution. Eighty-two shipments of radioactive substances were made during calendar year 1971.

TABLE V

RADIOLABELED MATERIALS PROCURED

NSC NO.	RADIOLABELED MATERIAL	LABEL
740	Methotrexate	H^3 (3',5')
755	Purine-6-thiol, monohydrate	C^{14} (8)
19893	Uracil, 5-fluoro-	C^{14} (2)
63878	Cytosine, 1-β-D-arabino-furanosyl-,	H^3
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-	C^{14} (Ring)
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-	C^{14} (Ethylene)
85998	Streptozotocin	C^{14} (Methyl)
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-	C^{14} (Ring)
102816	5-Azacytidine	C^{14} (4)
102816	5-Azacytidine	C^{14} (2)
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-	C^{14} (1)
129943	2,6-Piperazinedione, 4,4'-propylenedi-,	C^{14} (carbonyl)
132313	Galactitol, 1,2:5,6-dianhydro-	C^{14} (1)
139105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenoxy]-N,N-dimethyl-m-toluamide (1:1)	C^{14} (4,6)
404241	Adenine, 9-β-D-arabinofuranosyl-	H^3
409962	Urea, 1,3-bis(2-chloroethyl)-1-nitroso-	C^{14} (Ethylene)

During this report period the Section directed ten preparation laboratories (Table VI).

TABLE VI

CONTRACT PREPARATIONS LABORATORIES

FOR CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Aerojet Solid Propulsion Co.	Jagow	PH43-68-1300
Collaborative Research, Inc.	Friedman	PH43-66-506
Dow Chemical Co.	Love	NIH-71-2165
Merck and Co.	Babson	NIH-72-2002
Monsanto Research Corporation	Yanko	PH43-65-1044
Monsanto Research Corporation	Ellard	PH43-67-658
Stanford Research Institute	Henry	NIH-69-37
Stanford Research Institute	Tanabe/Leaffer	NIH-71-2162
Starks Associates, Inc.	Starks	PH43-62-484
Starks Associates, Inc.	Starks	PH43-68-1275

Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay and purity. All lots of bulk materials to be used in pre-clinical toxicological and pharmacological or in clinical studies are sent for analysis before the materials are released for use or formulation. Also, quality control data is obtained for all formulated materials prior to their release to the clinical investigators.

It should be pointed out that the Chemotherapy Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer

compounds has become involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the present of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultra-violet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research problem requiring the use of a combination of sophisticated techniques, and requires taking into consideration the stability of the compound and its possible contaminants or decomposition products.

In addition to the identification and analysis of compounds under study, the analytical unit is deeply concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. In certain situations the decomposition of a compound, its solution or formulation may be followed by the observation of the ultraviolet absorption when the decomposition of the active moiety results in a change in absorption. For the stability of compounds which cannot be followed by this procedure, other methods must be devised. Among the other procedures used are chromatography, determination of halide ion concentration, nuclear magnetic resonance, and mass spectrometry. To validate the accuracy of these procedures, determinations are made on a sample to which known standards have been added. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications".

The Unit collects and files such data as:

1. The detailed method of preparation;

2. The supplier's analytical data sheet; and

3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the Drug Development Branch directed quality control contracts.

The assembled data is of use also in writing purchase specifications for additional quantities of the compound. In general, attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1971, 71 compounds, representing 83 lots of bulk chemicals and 77 lots of clinical formulations, have been evaluated. Each of these evaluations is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicated unacceptable materials resulting in their being returned to the manufacturer for processing.

In addition, several special studies were performed. One analytical facility, Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. The procedure consists of equilibrating the compound in a mixture of n-octanol and water, and determining the concentration of the compound in each solvent. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities with their analytical and formulation problems concerning compounds of interest to the Chemotherapy Program.

The Section directed two analytical-quality control contract laboratories. In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE VII

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Midwest Research Institute	Haggerty	NIH-69-2113
Stanford Research Institute	Lim	NIH-71-2070

Miscellaneous

One contract, Pomona College (NIH-69-2112), is for structure-activity studies. This contract is directed towards (1) the use of the regression analysis techniques developed by Dr. C. Hansch of Pomona College to develop structure-activity correlations of antitumor agents and (2) the investigation of the factors which lead to the parabolic structure-activity relationship in enzyme-substrate systems which will be used to improve the quality of structure-activity predictions for drug-enzyme systems.

During this report period structure-activity correlations were investigated for five series of tests. The tests systems included the Walker 256, IP-L1210, IC-L1210, Lewis Lung and LD_{10} values. Optimum lipophilic character for a group of nitrosoureas was established when octanol-water partition coefficient (log P) values were correlated with LD_{10} values and antileukemic activity against IP-L1210 and IC-L1210. Optimum lipophilic character was also established for a series of triazeno-imidazole carboxamides when the octanol-water partition coefficient values were correlated with IP-L1210 antileukemic activity. These studies indicated that for the nitrosoureas, more hydrophilic nitrosoureas than have previously been prepared should be both more active and less toxic against both the IP and IC-L1210 test system. Based on these observations some nitrosoureas which would have the optimum lipophilic character have been designed and attempts to prepare them are underway. Work in this area of structure-activity correlations is continuing.

A detailed study was made of the physicochemical characteristics of the saturated phases of seven partitioning solvent systems and their relative values for use in structure-activity correlations were investigated. It was concluded that the octanol-water system was the best system. Comprehensive investigation of the parabolic structure-activity relationship in enzyme-substrate systems was continued.

TABLE VIII

MISCELLANEOUS CONTRACT LABORATORIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Pomona College	Smith	NIH-69-2112

CHEMICAL AND DRUG RECORDS SECTION
DRUG DEVELOPMENT BRANCH

SCOPE

The responsibilities of the Chemical and Drug Records Section are (1) to acquire chemical compounds for the primary screen and for other program needs, (2) to develop, maintain and update systems of registry for all chemicals entering the program and (3) to maintain adequate storage and inventory of bulk chemicals and drugs, and to coordinate the distribution of materials. These objectives are achieved through the concerted efforts by staff members to cultivate interest and to provide meaningful liaison with suppliers and potential suppliers; the surveillance of world-wide literature for new sources of chemicals and drugs; the development of chemical nomenclature for all compounds entering the program; the maintenance of Master Chemical Information files; the distribution and utilization of chemical data; the monitoring of contracts; and the assurance that all discreet compounds obtained from commercial sources are maintained in accordance with the signed agreements.

STAFF

During this report period the Section was staffed with five professional chemists, one biologist and one secretary. Of the professional staff members five are involved in accessioning of chemicals and drugs; the preparation of chemical information for computerized input and retrieval; and in chemical nomenclature. Two of these staff members are also concerned with the procurement of new synthetic chemicals and drugs for the primary screen. In addition to their assigned tasks the professional staff serves as project officers on various contracts that are maintained by the Drug Development Branch.

IMPLEMENTATIONS

Acquisition of Compounds

Materials entering the screening program for the most part are voluntary contributions from research investigators at universities, government agencies, various non-profit institutions, industries and individuals interested in the area of Chemotherapy. This continous voluntary input of samples for testing is enhanced by the exchange of information with suppliers through correspondence and personal visits of staff members to these various institutions.

To meet the expanding requirement for new materials by the Screening Program, the Section is (1) developing new sources of materials through personal contacts, literature surveillance and contact with the newly established NCI liaison offices in Brussels and Tokyo; (2) increasing the number of collection trips by staff members and by the initiation of a sample collecting contract; and (3) increasing the number of purchased compounds.

(1) Preliminary work done in the area of literature surveillance has shown that a large portion of the compounds compiled in the Current Abstracts of Chemistry would be new to the program. The results of these findings has dictated that efforts in this area be expanded.

Significant numbers of new sources of materials are located by personal visits of staff members to various research and academic institutions. The effectiveness of the personal approach is demonstrated by the number of large collections of compounds uncovered through these efforts. Over 1000 compounds from the late Roger Adams' collection were obtained in this manner.

The recent staffing of NCI liaison offices in Brussels and in Tokyo will provide additional means to develop new sources of materials from Europe and Asia. Surveillance for potential suppliers from these areas can be effectively monitored through these two offices.

(2) Previous experience has shown that suppliers do not transmit on their own volition sufficient numbers of new material for the primary screen. Therefore, additional samples must be provided through other mechanisms as collection trips, purchases and contracts, etc. During the past calender year 28 universities, 3 military research centers and 10 industrial sources were visited or contacted by DDB staff members. As a result of these visits 4,100 compounds were collected from academic research laboratories of which 3,272 were new to the program. The efforts with industry and with military research centers will not be reflected in this report since these sources will be submitting samples on a routine basis.

Compound collection trips affords the Program the opportunity to be more selective in the types of material that are acquired for screening. This is reflected in the continued lowering of the percentage of duplication for samples obtained from collection trips from 20% to 18%. Such trips also provide the opportunity for exchange of information with suppliers which often lends itself to the procurement of analogs of active compounds for evaluation. The screening results from such analogs may be useful for structure-activity correlation studies.

The Section is expanding its sample collecting capabilities by initiating a contract for this activity. The addition of this capability should provide many new sources and hopefully a significant increase in the number of compounds for testing.

(3) A review of our records revealed that the number of compounds purchased from chemical houses for evaluation has for many years remained at about 1.6%. Steps have been taken to increase the in-put of materials from this source.

The concerted effort in the procurement of samples for the screening program will be reflected in the actual numbers of compounds accessioned for the calender year of 1972.

For the calender year of 1971, a total of 10151 new synthetic compounds and crystalline natural products were accessioned, of which 881 were prepared under contract; 169 were purchased; 4034 were submitted by industry. Of the remaining 5067 compounds 3460 resulted from collection trips and the balance entered the program through voluntary submissions. The Chemotherapy Program has accessioned approximately 168,000 synthetic chemicals since its inception. Statistics for the total number of ring systems tested is not available, however, 13 new ring systems have been added to the active compound list. Active compounds have been found in 264 different ring systems.

CHEMICAL INFORMATION

The Section cooperated with PRA in the registering of the total chemical file for computerized information retrieval. All new submissions are registered and duplicate checks are made automatically. Significant support has also been provided by Staff members in the development of the new chemical information system under contractural arrangement with the University of Pennsylvania. For rapid retrieval of chemical information Master Chemistry Card Files and Molecular formula files are maintained.

In compliance with the order issued by the Food and Drug Administration, the Section monitors and maintains records of input and distribution of materials considered by FDA as narcotics, barbiturates, hallucinogenic and other dangerous drugs. The FDA has specified that separate and accurate records be maintained for these materials and only those individuals with the proper narcotic license be approved to dispense and receive these items. To insure proper safety, the drug abuse compounds are secured in a separate file under lock and key.

The confidential status of compounds obtained from industrial sources requires constant up-dating and careful monitoring. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationship but has made available many commercial samples not otherwise accessible. Frequently, there is a need to obtain the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agress, in writing, to declassify his compound.

CHEMICAL AND DRUG DISTRIBUTION

The Chemical and Drug Records Section is also responsible for the receiving, storage and distribution of chemicals for screening. The Section is charged with the custody of bulk chemicals and drugs that enter the Program. Detailed inventory records, such as source, date received, amount, lot number, initiator of all shipments and destination of drugs, are recorded on a daily basis.

The Section monitors the portion of the contract (PH43-68-1283) with Microbiological Associates, Inc. which handles the receiving, weighing, record-keeping, distribution and storage of compounds. Over 150,000 different materials are held in storage. These consist largely of chemicals and to a lesser extent plant products, antibiotic and steriods. A total of 8243 new compounds were shipped to the screening laboratories; 8605 refills and materials for special testing were weighed and 2072 packages were shipped. During 1971 approximately 16,000 samples were returned by the screening laboratories. These compounds, for which testing has been completed, are logged into the inventory.

TABLE XI

CONTRACTS MONITORED BY THE SECTION

Contractor	Project Officers	Contract Number
Chemical Abstracts Services*	Brown	PH 43-65-1021
Microbiological Associates Inc.	Berman	PH 43-68-1283
University of Pennsylvania*	Hill	NIH 71-2187

* Joint contract with Program Analysis Branch

CLINICAL DRUG DISTRIBUTION SECTION
DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the development, production, control and distribution of all investigational dosage forms of interest to the Chemotherapy Program. The Section is also responsible for the procurement and distribution of commercial formulations used by the Chemotherapy Cooperative Groups.

The responsibilities of the Section can be categorized as follows:

1. Development of new dosage forms for clinical use;
2. Production and packaging of investigational drugs;
3. Purchase of dosage forms;
4. Quality control surveillance of all clinical dosage forms;
5. Preparation of Pharmaceutical data for FDA-IND submissions, and
6. Storage and distribution of clinical dosage forms.

Staff

The staffing of this Section consists of three Pharmacists, one NIH Visiting Fellow, two shipping and receiving clerks and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing chemical and physical data and a review of in vivo route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned by this Section to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is provided with the route, dose level, and supporting available chemical data. Consultation with the Project Officer is carried out concerning feasible formulation approaches.

Routinely, drugs considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studies for the effects of pH, temperature, light and atmospheric oxygen. Additionally, compatibility tests are performed with buffers, preservatives and vial closures. Drugs considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some suitable parenteral dosage forms of potential antitumor agents. For this reason, the contract with the University of Michigan's Pharmaceutical Research Department is designed for exploring and developing new concepts of parenteral formulation of drugs with limited solubility and/or stability. A new contract was recently established with the Pharmaceutical Chemistry Laboratory, University of Kansas to further pursue research and development studies in pharmaceutical delivery systems.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses. The formulation data, screening and analyses results are evaluated by this Section for use by the Decision Network Committee. If the dosage form is acceptable, the compound is assigned by the Committee for toxicological assessment in dogs and monkeys.

The toxicological results and the accumulated pharmaceutical development data are distributed to members of the Dose Formulation Committee. These data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1971, 14 dosage forms were developed and seven of these entered clinical trial. An additional 15 dosage forms were modified from existing preparations.

Production and Packaging of Investigational Drugs

An important function of the Clinical Drug Distribution Section is to provide the Chemotherapy Program with sufficient quantities of formulated materials. The production of dosage forms is mainly carried out by contract agreement with qualified pharmaceutical manufacturers.

During 1971 the formulation contractors produced 9,785 bottles of tablets or capsules and 306,506 vials. This represents a 51% increase in units produced over the previous year. Three production contracts, Ben Venue Laboratories, Philips Roxane Laboratories and the National Drug Company, were monitored by this Section. A new contract with the Warner-Lambert Research Institute has been initiated as a result of the recent termination of the National Drug Company contract.

TABLE XII
CONTRACTS MONITORED BY THE CLINICAL DRUG DISTRIBUTION SECTION

Contractor	Investigator	Contract No.
Ben Venue Laboratories, Inc.	Wickes	PH43-66-909
Kansas, University of[1]	Higuchi	NIH 72-3217
Michigan, University of	Simonelli	PH43-66-1284
National Drug Company[2]	Scheindlin	NIH 70-2227
Philips Roxane Laboratories	Ebert	NIH 71-2139
Tennessee, University of	Avis	NIH 70-2084
Warner-Lambert Research Inst.[3]	Fand	NIH 72-3250

(1) Awarded March 1972
(2) Terminated November 1971
(3) Awarded April 1972

Some contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. This Section encourages these contractors to develop and produce their own dosage forms to relieve the workload of the formulation contractors. Specifications for formulation and production are provided to each contractor by this Section. This arrangement has proven economical, expedient and extremely beneficial to the program. Two contractors, The Upjohn Company (Contract PH43-68-1023) and Bristol Laboratories (Contract PH43-69-35), have provided formulated materials by this arrangement during 1971.

Several dosage forms are developed and produced by pharmaceutical manufacturers in cooperation with the Chemotherapy Program. By such an agreement the manufacturer supplies the dosage form free of charge or on a cost sharing basis in return for toxicological and/or clinical data. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between this Section and the manufacturer. During 1971, the following companies supplied a total of 110,574 vials and 2,212 bottles of tablets and/or capsules through this joint research effort.

COMPANY	DRUG
BRISTOL LABORATORIES, USA	NSC-125066, Bleomycin
FARMITALIA, ITALY	NSC-123127, Adriamycin
IVES LABORATORIES, USA	NSC-82151, Daunorubicin
ELI LILLY & COMPANY, USA	NSC-403169, Acronycin
MEAD JOHNSON LABORATORIES, USA	NSC-109724, Ifosfamide
MICROBIOLOGICAL RESEARCH ESTABLISHMENT, ENGLAND	NSC-106977, Erwinia Asparaginase
PASTEUR INSTITUTE, FRANCE	NSC-B-116117, BCG Vaccine
SQUIBB & SONS, USA	NSC-None, Hydroxyurea Placebo
THE UPJOHN COMPANY, USA	NSC-70735, Nafoxidine

Purchase of Dosage Forms

Several investigational dosage forms are procured by direct purchase from pharmaceutical suppliers. The product specifications are prepared by the Section and the final product is analytically tested by an independent laboratory and reviewed by the Quality Control Committee for Chemicals and Drugs before release for clinical use. During 1971, 10 investigational dosage forms were purchased involving 2,058 bottles of tablets or capsules and 40,827 vials.

This Section also obtains commercially available dosage forms for distribution to the Chemotherapy Cooperative Groups. These dosage forms are purchased either through the Veterans Administration Supply Contracts, or by special purchase through negotiated contract mechanism. All suppliers of commercial formulations are required to supply a certificate of analysis on each lot of drug purchased. The data is reviewed for compliance with strict manufacturing criteria. A total of 151,405 vials and 18,204 bottles of tablets and/or capsules were purchased during calendar year 1971.

Table XIII summarizes the procurement activities of the Section during calendar year 1971.

TABLE XIII
DRUG ITEMS PROCURED

SOURCE	CAPSULES	TABLETS	VIALS
Produced by Contractor	537,575	156,585	306,506
Contributed or Cost Sharing	24,600	130,920	110,574
Purchased Invest. Drugs	0	235,000	40,827
Purchased Commercial Drugs	40,000	1,681,400	151,405
	602,175	2,203,905	609,312

TOTAL: 3,415,392 units

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, in collaboration with the Chemical and Drug Procurement Section, maintains a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator and sometimes by another laboratory.

Individual unit assays are required; the number of samples assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an outside analytical contractor to verify the analytical methodology and accuracy of the formulator. The confirmation analyses are mainly performed by Stanford Research Institute and Midwest Research Institute.

This Section participates in the activities of the Quality Control Committee for Chemicals and Drugs. This committee reviews for clearance the analytical results of bulk chemicals and formulated clinical dosage forms. During calendar year 1971, 175 lots of clinical dosage forms were reviewed by the Quality Control Committee for Chemicals and Drugs. Of these, 116 were investigational dosage forms and 59 were commercial dosage forms.

The Pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under similated clinical conditions to test the effects of temperature, light and salt additives. The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf-life surveillance program on all lots of noncommercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Two assay schedules are used for shelf-life studies: Schedule A, designed to provide stability data under accelerated conditions; and Schedule B, designed for controlled studies of repeat formulations where adequate stability data is available. Both schedules provide a two-year surveillance record.

Preparation of Pharmaceutical Date for FDA-IND Submissions

This Section prepares attachments I, II, III, V and VII for submission to Cancer Therapy Evaluation Branch for IND filing with the Food & Drug Administration. These attachments consist of the description composition, method of manufacture and controls used in the preparation of the dosage form. The FDA requires that such a submission be made for each new investigational drug prior to clinical release. In addition, IND amendments must be submitted whenever a new dosage form of an existing IND drug is formulated for clinical use.

During 1971, this Section prepared 5 original IND reports and 31 amended IND reports.

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities of the Section are carried out by two shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year. During 1970, approximately 539 foreign drug shipments were made.

During 1971, a total of 642,021 drug items (vials and bottles) were received and inventoried for subsequent world-wide distribution. During that same period, 560,265 drug items were distributed (30% increase over 1970) averaging 32 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs; the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by a supply clerk and checked by the pharmacist prior to shipment. There are presently 19 such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory kardex file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on the same card by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgement to restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors.

During 1971, the Section had an average inventory of 325 dosage forms by lot number involving 186 different formulations of 109 different compounds. These figures include investigational and commercial dosage forms.

Serial No.: 3584
1. Chemotherapy, DR&D
2. Drug Development Branch
3. Clinical Drug Dist'n Section
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Research in Dosage Form Development of New Antitumor Drugs

Principal Investigator: J. Paul Davignon

Other Investigators: Kim W. Yang, James C. Cradock, John S. Driscoll and Harry B. Wood, Jr.

Man Years

Total: 1.25
Professional: 1.25

General Objectives:

The objectives of this project are to:

(1) Study new formulation approaches with potential utility for those compounds posing difficult formulation problems.

(2) Modify existing parenteral formulations to yield preparations more physiologically compatible with cerebrospinal fluid.

(3) Evaluate dosage form problems presented by clinical investigators.

Specific Objectives:

(1) Development of a parenteral dosage form for NSC-95441 (Methyl CCNU) and for NSC-79037 (CCNU).

(2) Modification of the available parenteral form of NSC-32065, (Hydroxyurea) for toxicological evaluation by the intrathecal route of administration.

(3) Modification of the available parenteral form of NSC-77215 (Procarbazine) for toxicological evaluation by the intrathecal route of administration.

(4) Development and formulation of stable parenteral preparation of NSC-4728.

(5) Formulation of a more suitable parenteral dosage form of NSC-71795 (Ellipticine).

Major Findings:

I. Development of Parenteral Forms of Methyl CCNU and CCNU. A 90% aqueous parenteral form of Methyl CCNU and CCNU has been developed.

Methyl CCNU and CCNU do not display the same solubility in hydro-alcoholic solutions as BCNU (NSC-409962). Therefore a new approach to the design of a parenteral dosage form was needed. A preparation containing NSC-95441, in absolute ethanol and polyethoxylated vegetable oil was found to be miscible with distilled water, 5% Dextrose Solution or Normal Saline Solution. A similar preparation was made with the CCNU. The vehicle was found to interfere with the U.V. spectrophotometric analysis of the parent drug and therefore a gas chromatographic method is under development.

II. Design of Dosage Forms for Intrathecal (I.T.) Administration.

Drug formulations intended for intravenous administration frequently contain additional agents to enhance stability or solubility. Due to rapid dilution and buffer capacity of blood these additives are usually pharmacologically inoccuous. However, these same formulations may not necessarily be administered intrathecally with the same margin of safety due to cerebrospinal fluid (CSF) volume or CSF buffer capacity. A toxicological assessment of intrathecally administered NSC-32065 (Hydroxyurea) and NSC-77215 (Procarbazine) was desired. Since parenteral formulations of both agents contain additives (buffers, chelating agents, or antibacterial preservatives) formulations containing Hydroxyurea or Procarbazine alone were desired.

A lyophilized formulation of Hydroxyurea was developed and produced that provided 100 mg/ml Hydroxyurea at pH 7.2-7.4. A gas chromatographic method for analysis of this compound was also developed.

Procarbazine for parenteral use is formulated as the HCl salt and aqueous solutions exhibit a pH of about 2.85. Formulations of this compound at higher pH ranges (6-7) were soluble but quite susceptible to oxidation. Development work indicated that a stable lyophilized product could be prepared. Stability was enhanced by the addition of 0.1% sodium bisulfite.

Intrathecally administered Methotrexate is a recognized method of treating meningial leukemia. Neurological toxicity has been reported following I.T. administration of Methotrexate to certain patients. The commercial formulation also contains the antibacterial preservatives Methyl Paraben and Propyl Paraben. A sterile formulation containing these parabens in Elliott's B solution was prepared for the toxicological evaluation in monkeys of I.T. administered parabens.

III. Formulation Development of 1,3,4 triadiazole,2-amino (NSC-4728).

This drug was successfully formulated as a lyophilized parenteral dosage form. The water solubility of NSC-4728 was increased 10 to 12 fold by preparing solutions at pH 2-3. Toxicological studies are being conducted.

IV. Formulation of Ellipticine (NSC-71795).

Ellipticine (NSC-71795) as the HCl salt was reported to be hemolytic *in vivo* and *in vitro*. Hemolysis was abated by addition of citrate buffer (Lee, I.P. and Dixon, R.F., Fed. Proc. 31:544, 1972). A formulation of Ellipticine in pH 3.3 citrate buffer was prepared for antitumor evaluation.

Miscellaneous Projects:

The availability of a dosage form development laboratory has enabled a number of small projects to be carried out in cooperation with other groups. Some short term high priority projects were also performed as needed in the laboratory.

Publications: None

NATURAL PRODUCTS SECTION

DRUG DEVELOPMENT BRANCH

SCOPE

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from microbial sources, higher plants and animal products. This area will be discussed under the headings of Fermentation Products Program and Plant and Animal Products Program.

Staff

This Section has operated with a staff of four persons, consisting of two professional (Ph.D. level) and two clerical employees.

Surveillance

World-wide surveillance of the published literature and collection of chemicals and drugs for biological evaluation are carried out in the Fermentation Products area. It has not been possible to undertake surveillance in the Plant and Animal Products area.

Statistical Summary

Table I lists the natural product materials acquired during the year under review compared with the previous year. Of the crude extracts, contractors provided practically all of the 1215 fermentation products, 80% of the 1203 animal products, and 56% of the 3015 plant products.

FERMENTATION PRODUCTS PROGRAM

Since the isolation of penicillin in the early 1940's, microorganisms have been an important source of new chemotherapeutic agents in the infectious disease area, and we believe they have as great a potential in cancer chemotherapy. Three groups of organisms have been shown to be the principal producers; they are the bacteria, actinomycetes, and fungi. Antibiotics produced by these microorganisms have shown a wide range of activity and chemical structure and have introduced a number of new structure types to the chemical literature.

There have been approximately 290 highly purified fermentation products collected world-wide which have been reported to be active *in vivo* against one or more animal tumor systems. An additional 64 are reported active *in vitro* against tumor cells in tissue culture. This latter group is also sometimes referred to in the literature under the general term "Antitumor active". Of these two groups, 144 of the *in vivo* actives and 24 of the *in vitro*-only active group have been entered in our program. Eighty-six of the 144 *in vivo* actives have met our criteria for activity in one or more of the tumor systems used in our program. Many did not show activity great enough to warrant further development. Thirty-six of these have been in

clinical study up to the present and 12 are still under clinical investigation. Those now in clinical study are Actinomycin D, Adriamycin, L-Asparaginase from Escherichia coli, Azaserine, Azotomycin, Bleomycin, Daunomycin, Mithramycin, Mitomycin C, Porfiromycin, Streptonigrin, and Streptozotocin. Other fermentation products in clinical study in other parts of the world include: Chromomycin A_3, Neocarzinostatin, Olivomycin A, Actinomycin C, Anguidine, and L-Asparaginase from Erwinia carotovora. All but Actinomycin C are under development in our program.

The antitumor fermentation products program has been carried out through contracts at a number of different biological laboratories, each of which used its own approach to soil collections, culture isolation, selection of culture types, fermentation development, and chemical isolation. Some of the contracts in this area have differed from the synthesis and plant product contracts in that a sizable amount of the effort was devoted to in-house screening and other assay services.

The Section monitors several fermentation contracts. These are listed in the accompanying Table IX. The reduction in number of contracts, during the past several years, has had a marked effect on the number of agents being fractionated and on the number of purified samples received for pre-clinical evaluation.

TABLE IX

CONTRACT LABORATORIES FOR FERMENTATION PRODUCTS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Dr. Bradner	PH43-69-35
Michigan Department of Health	Dr. Olson	PH43-65-7*
The Upjohn Company	Dr. Fonken	PH43-68-1023

*This contract provides a tissue culture assay laboratory for a fermentation program supported by the State of Michigan.

During the past year, the primary screen in the Upjohn contract was changed from one selecting antimetabolites to a routine cell cycle specificity screen using synchronous cells in culture. Compounds showing activity in any sectors of the cell cycle are to be given top priority in development.

Emphasis continues on the screening of fungi though many actinomycetes are still included. During the year, four new purified materials have been received for evaluation from the contract laboratories. Studies on these are still in progress.

Four agents passed Decision Point 2 during this period. These include Rifamycin SV, D,L-Alanosine, Anguidine, and (Mer) BCG. Toxicological studies are continuing on Chromomycin A_3 and Neocarzinostatin and will begin on

Anguidine as soon as a satisfactory dosage form is available. The progress of the antibiotic Poricin has been delayed primarily due to the inability to prepare a stable product. This now appears to be solved and animal studies with the stable material are in progress. A sufficient quantity is available for toxicology and Phase I studies. IND's have been filed on Rifamycin SV and 5-Azacytidine during this year. The latter was originally found in fermentation studies but is now prepared synthetically.

The fermentation and chemical literature continues to be reviewed for new antibiotics and other metabolic products which should be screened against our in vivo systems. As a result of this review, 17 purified and 48 crude samples were received from world-wide sources for screening. Nine purified samples were received from our contractors. The total number of purified fermentation products for the year is 26 and these have all been submitted for screening.

PLANT AND ANIMAL PRODUCTS PROGRAM

In carrying out its objectives in the plant and animal products area the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (a) plant and animal collecting; (b) preparation of extracts; (c) fractionation of active extracts and isolation and identification of the active agents; and (d) in collaboration with the Chemical and Drug Procurement Section in the preparation of large amounts of active agents for preclinical toxicology and clinical trial.

Through extensive correspondence, contact is maintained with potential domestic and foreign suppliers in order to acquire plants, animals, and pure compounds of natural origin which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

During the current year it was possible, through increased funds, to initiate one new contract for the procurement of animal products, preparation of extracts and fractionation of those extracts found to be active, and another new contract for the fractionation of active plant materials (see Table X). In addition, our three contracts for the supply of plants were increased substantially. Because of the increasing necessity in recent years to re-collect large amounts of natural products for fractionation, and the reduction in dollar amount of the contracts, the general collection of new plants under contract has substantially decreased from a normal level of approximately 5000 per year to about 3000. This has had an adverse effect on the number of pure compounds coming out of the program. Other factors causing a drop in the number of pure compounds are (a) the discontinuance of fractionation work during and since FY 1971 on plants active in Walker 256 (WM) and other tumors, and (b) the substantial backlogs at the screening contractors until the fall of 1971. During the year under review one of these factors has been eliminated and the other minimized--new accessions have been increased and backlogs at the screeners have been reduced.

The following contractors are engaged in the Plant and Animal Products program:

TABLE X

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Arizona, Univ. of	Brewer; Cole	PH43-67-1484
Arizona State Univ. 1/	Pettit	NIH-71-2308
Bristol Labs. 2/	Bradner	PH43-69-35
Central Drug Res. Inst.	Dhar	01-004-1 (PL-480)
Costa Rica, Univ. of 3/	Saenz R.	PH43-64-31
Hawaii, Univ. of	Sagawa	PH43-67-748
Illinois, Univ. of 4/	Farnsworth	NIH-NCI-C-72-2078
Midwest Res. Inst. 2/	Cheng	PH43-65-94
Research Triangle Inst.	Wall	NIH-69-2019
Southern Res. Inst. 2/	Montgomery	PH43-64-51
U.S. Department of Agriculture	Perdue	NCI-CR-64-1 (Transfer)
Virginia, Univ. of	Kupchan	PH43-64-551
WARF Institute, Inc.	Derse	PH43-67-747

1/ Effective June, 1971.

2/ Minor part of the total contract.

3/ Terminated in December, 1971, and renewed under an open-end contract.

4/ Effective March, 1972.

In addition, an open-end, fixed-price contract for the procurement of plant extracts has been put into effect with Northeastern University (Dr. R. E. Raffauf).

Beside the contractors listed above, several groups including grantees and those not supported by the NIH contribute in different ways to this program without cost to DR&D. The major groups are: C.S.I.R.O. (Australia), U.S.D.A. (pesticide Chemical Research Branch, Beltsville), U.S.D.A. (NURDD, Peoria),

C.S.I.R. (South Africa), National Defense Medical Center (Taiwan), Purdue University (Dr. Cassady), Virginia Polytechnic Inst. (Dr. Kingston), Univ. de Concepcion, Chile (Dr. Silva), and Univ. of Oklahoma (Dr. Weinheimer).

Progress was made in these areas of natural products as follows:

A. Marine organisms

The new contractor (Arizona State University) is responsible for the procurement of the raw materials. At present, 24 different species are under fractionation by all fractionators and one crystalline compound of marginal activity in PS has been isolated. The current rate of about 1200 new extracts per year is being maintained.

B. Plants

1. General collections. While 3015 new extracts were accessioned during the year under review, the current rate of collecting has been increased to about 10,000 per year. It is projected that the number of submissions for fiscal 1972 will be 5000 and for both calendar year 1972 and fiscal year 1973 will be 10,000. The U.S.D.A. has scaled up its general collecting from a rate of 1000 per year at the beginning to 8000 per year at present; the University of Hawaii has increased its general collecting to a rate of over 500 per year; the University of Costa Rica has doubled its collecting to a rate of over 400 per year and, at their request, have terminated their regular contract in favor of an open-end, fixed-price contract. The remaining extracts are accounted for by input from miscellaneous sources.

   Improvements have been made in our plant collection procedures which will ultimately increase the yield of active agents and shorten the time lag in obtaining active agents. Continuing analyses have shown that certain families and genera yield a relatively higher proportion of active agents, while others yield a low proportion. Where possible, more intensive collections of "rich" families and genera are carried out, while the "poor" families are avoided.

2. Large re-collections. These are of two kinds: 25-50 lbs. for fractionation work and 500-30,000 lbs. for isolation of large quantities of active drugs needed for preclinical toxicology and clinical trial. The former have been increased in number to over 200, and the decision has been made to increase the quantities to 50-100 lbs. or 150-200 lbs. where possible. Four very large re-collections have been made and are either undergoing or awaiting processing. These are: Acer negundo (for Acer saponin) from Wisconsin, Heliotropium indicum (for indicine N-oxide) from Taiwan and Brazil, Maytenus ovatus (for maytansine) from Ethiopia, and Tripterygium wilfordii (for an LE-active alkaloid) from Taiwan. In addition, steps have been taken to increase the supply of the plant Cephalotaxus harringtonia (for harringtonine) by establishing large-scale cuttings at the U.S.D.A. Plant Introduction Station in Georgia and at different nurseries.

Also, steps have been taken to increase the supply of Thalictrum dasycarpum (for thalicarpine) by obtaining and analyzing different strains of seeds, and conducting experiments on best methods of growing the high-alkaloid strains. Both the Cephalotaxus and Thalictrum are not plentiful enough in their native state to be adequate sources of their respective active agents if the latter should prove useful.

3. Duplicate searching: Automated procedures have been developed with the Program Analysis Branch which are making possible a more efficient and economical accessioning and testing of plant and animal materials. This concerns a search for "duplicates". Using a typewriter-type terminal which communicates telephonically with a computerized data base containing 60,000 plant names, a typist types the first three letters of the genus and species names to be checked as duplicates and is immediately informed whether or not the names match names on a list of "duplicates". The latter is a list of names of confirmed active plants and of those we have had ten or more times, which we do not wish to accession again for testing. Hitherto, using a manual search of a header card file, we could afford to search only about 2000 names a year. With the present improvement we are handling our present input of about 10,000 per year and will be able to take care of any foreseeable increase. The saving in extraction and screening should be substantial.

4. Decision Point 2 and IND materials: Three materials passed Decision Point 2--coralyne (NSC-96349), 12,13-dihydro-13-methoxynitidine (NSC-146,396) and nitidine chloride (NSC-146,397). In addition, one semi-synthetic compound--epipodophyllotoxin, 4'-demethyl-,4,6-O-ethylidine-β-D-glucopyranoside (NSC-141,540)--passed Decision Point 2. An IND was filed on thalicarpine (NSC-68075) but was later withdrawn for additional toxicological studies.

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ANNUAL REPORT

DRUG EVALUATION BRANCH

DRUG RESEARCH AND DEVELOPMENT

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

Principal objectives of the Drug Evaluation Branch (DEB) are to initiate, develop, and coordinate laboratory programs for recognition, recommendation, and development of new drug therapies against human cancer. Objectives are achieved by (a) screening large numbers of synthetic and natural products for biological activities suggesting potential clinical usefulness, (b) evaluating "active" drugs with respect to conditions of usage (e.g., treatment route and schedule, combination chemotherapy, surgery plus chemotherapy) which may enhance therapeutic efficacy leading to cures of animal tumors, (c) determining drug effects on host immune responses (suppression or enhancement) and their implications to therapy, (d) studying experimental means of altering antigenicity of tumor cells and thus their sensitivity to chemotherapy, (e) investigating mechanisms of drug action and resistance, (f) developing new and improved methods for drug evaluation, and (g) investigating related specific problems arising during preclinical toxicological examination, clinical formulation development, and clinical trial. Studies are aimed at providing information essential to achieving objectives as defined in the Chemotherapy Program's Linear Array, at research contributing directly to the development of methods for providing such information, and basic research of significance to understanding of relevant scientific principles that could have a major impact on program objectives. Program is implemented through contracts with qualified institutions and through intramural laboratory projects.

DEB includes the Office of the Chief (J. M. Venditti) and three sections: Biochemistry (F. R. White, Head), Immunotherapy and Immune Response Modifiers (IIRM; A. Goldin, Office of the Associate Scientific Director, DR&D, Principal Advisor), and Screening (B. J. Abbott, Head); and is staffed by 12 senior professionals (including 2 staff fellows, 2 staff associates, and 2 commissioned officers) and 12 junior professional, technical, and clerical employees. In addition, staff is supported by two part-time employees and four aids under work-study plans.

Staff have primary responsibility for 16 contracts funded at approximately $7,000,000 plus three PL-480 agreements, and responsibility for drug evaluation related activities carried out under four multifaceted contracts funded at $6,000,000 and one PL-480 agreement administered by other Chemotherapy units. In addition, staff (a) review bioassay results and select natural products for collection, fractionation and isolation of active components under six Drug Development Branch contracts; (b) advise the Etiology Program of candidate drugs for carcinogenesis studies; and (c) collaborate with the Program Analysis Branch and Office of the Chief, DR&D with regard to automatic data retrieval and processing needs and

animal needs, respectively. The relationship of DEB-monitored contract and intramural projects to activities outlined in the Chemotherapy Program Linear and Supplementary Arrays and to the overall objectives and approaches of the National Cancer Plan are listed in Table I, parts A, B, and C.

Comprehensive drug evaluation programs were continued at Southern Research Institute (SRI), A. D. Little, Inc. (ADL), and Microbiological Associates, Inc. (MAI), where tasks were devoted principally to detailed evaluation of drugs in development toward clinical trial, conduct of therapeutic trials in animals leading to cures, and development of new screening assays. Tasks conducted under DEB-sponsored contracts with SRI (71-2098, 66-29, and 71-2189) were integrated with SRI projects related to pharmacology and physiological distribution of active drugs (65-654 and 70-2083) and synthesis of new agents (71-2021). The Drug Evaluation Division and Immunology Division, MAI (68-1283) represent collaborative laboratory resources for DEB staff who participate in an advisory capacity in day-to-day evaluation of tasks (see Project Reports, Serial Nos. NCI-3576 and 3577). In addition, basic and developmental studies are conducted in-house under the Office of the Chief, DEB (Project Report, Serial No. NCI-3583), the IIRM Section (Project Report, Serial No. NCI-3577) and the Screening Section (Project Report, Serial No. NCI-3578).

During calendar year 1971, 15,584 synthetic and natural products were submitted to DR&D for primary screening. One-hundred-ninety-five showed "confirmed minimal activity" in initial screening. The Drug Evaluation Committee reviewed screening results in detail for 462 agents and recommended 17 to Chemotherapy Staff as candidates for Decision Network (DN) 2A. In all, 19 agents passed DN-2A during 1971. These and their selection bases are listed in the current organizational report of the Screening Section.

During 1971, total in vivo screening capacity was increased from a level of approximately 120,000 tests/year to the current rate of approximately 320,000 tests/year. The latter level was projected as required to accommodate an increased input of materials into our program and the addition of new screening assays. Two new primary screening contracts were added during 1971, bringing the current total to five. The 320,000 tests/year level was reached by progressively increasing testing levels within existing contracts (the five primary screeners plus SRI, ADL, and MAI). All primary screening contracts were recompeted during 1971, and we do not foresee a need for additional contractors in this area in the immediate future.

Leukemia L1210 remains the basic assay for primary screening of synthetic materials. Analysis of schedule dependency data over the last two years indicated that daily treatment and intermittent treatment (q4d) were the schedules most likely to minimize the number of false negatives, and these schedules are used routinely whenever the supply of material permits. Analysis of plant product screening results against L1210 and P388 showed that the L1210 system provided an insignificant number of active leads among crude plant extracts. In contrast, a significant number of crude plant extracts showed initial activity against P388, and approximately 8% of the latter demonstrated L1210 activity after fractionation or isolation of the P388 active component. As a consequence of this finding and previous studies

showing that P388 and L1210 respond to the same kinds of drugs (with the exception of purine analogs), but that P388 is the more responsive tumor quantitatively, natural products are now tested against P388 on two schedules (q4d and qd). Those found active in the P388 system are then tested against L1210 after partial fractionation.

Despite our considerable success in devising experimental systems that predict well for the treatment of human acute leukemias and lymphomas, we still need to improve our score against these more rapidly proliferating diseases. Encouraged by recent clinical successes using drug combinations, efforts devoted to this important approach to curative therapy have been increased. Combination drug therapy has been more successful than single drug treatment against a number of tumors of man and animals. The effectiveness of the combination of Vincristine plus prednisone against spontaneous AKR leukemia has been well established. A list of 40 drug pairs that demonstrated therapeutic potentiation against L1210 leukemia are included in the current organization report of the Screening Section. Most of these studies were conducted at SRI and MAI. Following the previous finding of therapeutic potentiation of 6-thioguanine (6-TG) and cytosine arabinoside (Ara-C) attributable to the reduction of toxicity of one antitumor agent (6-TG) by another (Ara-C), at least two other equally notable combinations have been found. The anti-L1210 drug, ICRF-159 has been found to block the toxicity of Daunomycin, and the combination of ICRF-159 + Daunomycin provided a high degree of synergism against L1210. N-Acetylcysteine, (NAC), inactive as an antitumor drug, protected against the toxicity of I-phosphamide. The combination of NAC + I-phosphamide, including normally supralethal dose of I-phosphamide when given alone, was curative in 100% of mice with L1210. A major problem in an overall program to study drug combinations has been the rationally based selection of drug pairs to be tested. Recently, the group at Southern Research has prepared a reference chart outlining patterns of resistance and cross-resistance among 27 useful and interesting anticancer agents. It is expected that such analyses will be of practical usefulness in the rational planning of combination chemotherapy trials in animals.

Emphasis on the studies of treatment schedule dependency as a means for improving responses to active drugs is continuing. A comprehensive review of the results of DR&D schedule dependency studies against L1210, their implications for screening methodology, and for the design of toxicological and clinical trials has been published (Venditti, CCR, part 3, 2: 35, 1971). There is ample evidence to support the following conclusions. There is no single optimal schedule for all drugs. Schedule dependency as well as anti-tumor specificity depends on the inherent sensitivity of the tumor and critical host cells, the size of the tumor cell population, the proliferative state of the tumor, the pharmacological distribution and metabolism of the drug, and the biochemical site of drug action. Schedule dependency cannot be predicted on the basis of chemical structure except in some cases where specific sites of biochemical action can be successfully predicted. Therefore, active analogs of interesting drugs should be accorded a moderate measure of schedule dependency testing. For example, a number of analogs of Ara-C have shown patterns of schedule dependency different from the parent compound. Thus, the insoluble palmitate ester of Ara-C acts as a "depot" form of the parent compound and is as effective as a single treatment as is Ara-C given

q3hr., q4d. 0-2,2'-cyclocytidine (Anhydro-Ara-C), a water soluble analog, is as effective given daily as is Ara-C given q3hr.,q4d. It is possible that Anhydro-Ara-C which appears to be relatively resistant to deamination in vivo may break down to Ara-C slowly and thus represent a soluble "reservoir" for the parent compound. The schedule dependency of a drug given parenterally may or may not be retained when it is given orally. The disease state at the initiation of treatment can alter a drug's schedule dependency. Thus, against advanced L1210, Ara-C given once every second or fourth day is as effective as q3hr.,q4d. treatment. Moreover, reduction of the tumor cell population by an initial Cyclophosphamide treatment partly restored the early tumor-like schedule dependency of Ara-C against advanced L1210.

Cytokinetic implications for the success of therapy have become apparent for both animal and human cancers. Many of our useful drugs kill cells because they inhibit biochemical events peculiar to cell division, i.e., DNA formation and function. Thus, recent successes in the clinic have involved the treatment of relatively rapidly proliferating diseases. However, we need to develop experimental systems that predict useful treatment for slower-growing human cancer; especially breast, lung, and colon. An approach to this problem has been to attempt to develop models that simulate these diseases with respect to cytokinetics. Accumulated data on tumor growth kinetics (mainly from SRI) indicate that the cytokinetic characteristics of rapidly-growing tumors are (1) a relatively high proliferative fraction among viable cells, and (2) relatively little cell loss during growth. In contrast, more slowly-growing tumors are characterized by (1) a lower proliferative fraction among viable cells, and (2) greater cell loss. With the slower-growing tumors, doubling time (Td) increases with mass. The increase in mass and Td is accompanied by a progressive decrease in proliferative fraction. The degree of response to chemotherapy of a wide variety of "fast" and "slow" growing tumors is inversely related to the advancement of the disease. Thus the efficacy of therapy is related to both the "kinetic" state of the tumor and the total tumor cell population as well as to other variables that influence sensitivity to drugs. A significant variety of animal tumors have been "cured" if chemotherapy was initiated early or after surgical removal of the larger and less rapidly growing solid tumor mass. The SRI, ADL, and MAI groups have determined the growth and operational characteristics of a number of additional (to L1210) experimental tumors including DMBA-induced breast, C3H breast, AKR lymphoma, B16 melanoma, and Lewis lung carcinoma (LL). Application of the knowledge gained in these studies has resulted in significantly improved therapeutic response of these tumors to available drugs. For example, therapeutic potentiation of alkylating agents in simultaneous combination was demonstrated against SC-implanted LL, B16, and 1st generation transplants of C3H breast with Cyclophosphamide plus Me-CCNU (NSC-95441). This combination has also been shown to significantly increase the "cure" rate of mice bearing LL when used as adjuvant treatment following non-curative surgical removal of advanced primary tumors. Several demonstrations of therapeutic potentiation of cyclophosphamide plus a number of nitrosoureas (BCNU, CCNU, Me-CCNU) against slower-growing transplantable tumors have been the basis for ongoing or planned clinical trials with such drug combinations. Drug responses of B16 and C3H adenocarcinoma to a number of active anticancer drugs has been determined. Application of non-cell cycle stage specific plus specific drug therapy or chemotherapy plus surgery has resulted in improved

treatment of these largely refractory tumors. B16 melanoma appears to show sufficient reproducibility with respect to growth characteristics and response to treatment to be useful as either a primary or secondary screen now. Recently, a project was initiated in which all primary screening laboratories will test a number of selected active and inactive drugs twice each to quantitate the criteria for use of IP-B16 as a screen and determine test-to-test and lab-to-lab reproducibility of results. The study is similar to that conducted with L1210 some years ago. "The L1210-Model Screen", which aided immeasurably in establishing protocols for screening and criteria for acceptance. At the same time, two laboratories are conducting parallel studies against SC-B16 inasmuch as the site of implantation appears to influence the response of B16 to treatment for a number of interesting drugs, particularly Daunomycin, Adriamycin, and Bleomycin. Recently, the SRI group has developed protocols for screening against early transplant generations of spontaneous C3H carcinoma and has determined that the kinetics of growth of the early transplant generations does not differ in a major way from those of the spontaneous tumor. Consequently, we have instituted a well-defined project for the limited use of this tumor for detailed study of a number of drugs of clinical interest specifically in the therapy of human breast carcinoma. The LL tumor has shown more variation in test-to-test drug sensitivity than we would like to see if we anticipate using it as a primary screen. Nevertheless, LL has been an important tool in uncovering new agents worthy of clinical trial (e.g., Me-CCNU) and in the detailed evaluation of known drugs and combinations. Drugs which inhibit the local tumor growth severely when given early or cause substantial regression of established LL do produce measurable and reproducible increases in survival. However, there has been little positive correlation of therapeutic efficacy as manifested by increased lifetime with moderate (<75%) local tumor weight inhibition. The methodology for use of LL as a primary or secondary screen, and indeed, its desirability as a primary screen are still under study. Neither B16 nor LL (even in the advanced state) demonstrate the very low proliferative fraction that would mimic advanced slow-growing clinical tumors. However, considering their growth characteristics, responses to known drugs, and feasibility of use for testing large numbers of agents, they were considered the experimental tumors of choice for secondary screening at this point in time. At the same time, we are continuing investigating other assays at the basic level; e.g., P815 mast cell tumor which may exhibit a long Td when inoculated SC and a number of osteogenic sarcoma models. In our concerted effort to foster collaboration among all in vivo screening laboratory investigators in aiding in problem solution and advising program, we raised the questions of primary and secondary screening as the major topics for discussion at "Screening Workshops" held on November 4, 1971 and on April 14, 1972. Hopefully, direct discussions among NCI staff, laboratories devoted to new assay development, and laboratories who actually conduct the bulk of our in vivo screening will aid further in the development of an expanded large-scale screening program that will play a major role in attaining the goals of the National Cancer Plan.

Projects related to the characterization and treatment of AKR-lymphoma are being conducted under contract at SRI, MAI, and IITRI (contract 68-1021) and in-house within the IIRM Section (see Project Report, Serial No. NCI-3577) and Screening Section (see Project Report, Serial No. NCI-3578). Determinations of the biological characteristics and growth kinetics of spontaneous AKR

lymphoma were extended, and single drug and combination treatment of this disease have been carried out. With therapy beginning at the time of diagnosis, significant increases in host survival were obtained by optimal regimens of Ara-C, Palmo-Ara-C, 5-FU, Vincristine plus Prednisone, Daunomycin, Cyclophosphamide, and selected nitrosoureas. Optimal combination therapy of spontaneous AKR lymphoma has apparently achieved eradication of all clonogenic tumor cells in some mice. However, apparent "cell cure" of this disease (in which the tumor and host cells carry the RNA oncogenic virus) appears to be followed by reinduction of a second lymphoma cell population. This same reinduction process was observed after "cell cure" of transplanted AKR lymphoma in young AKR mice. Treatment of spontaneous AKR lymphoma with Ara-C or Palmo-Ara-C resulted in increases in lifespan but no cures. Tumor cells isolated just prior to death from these treated mice are up to 100 times less sensitive to Ara-C than previously untreated spontaneous AKR lymphoma cells. This and results for the Ara-C treatment of L1210 (see Project Report, Serial No. NCI-3576) suggested that emerging resistance to Ara-C may be a constant obstacle to curative therapy with this drug. It is noteworthy that resistance of L1210 to Ara-C occurred while normal bone marrow cells retained their sensitivity to the drug suggesting the need for increased emphasis on combination chemotherapy. It has been shown that L1210 effectively treated but not eradicated by the synergistic combination of Ara-C + DIC (NSC-45388) became refractory to Ara-C, and partially refractory to DIC but remained sensitive to MTX or BCNU. Such observations can be extended to the rational planning of therapeutic regimens against spontaneous AKR lymphoma, in which protocols have been designed to study remission maintenance following successful remission induction. During the year, Dr. Emil Frei (M. D. Anderson) reported the findings of the AKR subcommittee of the Chemotherapy Ad Hoc Advisory Group. Having reviewed existing data relative to the spontaneous AKR program, the subcommittee concluded that this system (and the Moloney virus-induced leukemia in BALB/c mice) is a promising tool for detailed evaluation of a limited number of drugs of interest. Results of therapeutic trials of spontaneous AKR lymphoma are scanty except for detailed studies of a very limited number of drugs. This problem is being alleviated to some extent by the increased availability of AKR mice, but the logistical problems inherent in the use of the spontaneous AKR lymphoma (long animal holding time, the need for continual surveillance of the entire holding population for appearance of disease, the need to enter animals on test individually) obviate its use for secondary screening of even a moderate number of drugs. The SRI and IITRI groups have studied the growth characteristics of first passaged AKR lymphoma. In contrast to long-passaged lines which exhibit growth characteristics similar to L1210, 1st passaged AKR lymphoma, in the advanced state, appears to approach the growth kinetics of the spontaneous disease. Relatively few animals with spontaneous disease can yield sufficient leukemic thymic or splenic tissue to implant a large controlled population of AKR mice with homogeneous 1st generation AKR lymphoma. A protocol for testing relatively large numbers of drugs against 1st passaged AKR lymphoma has been developed at IITRI and in-house and a number of therapeutic trials against early and advanced disease have been run (Project Report, Serial No. NCI-3578). Hopefully, the 1st passage AKR system will provide a useful prescreen for the rational selection of new therapies including drug combinations for trial against spontaneous AKR lymphoma.

Natural products continue to be screened against KB cells in culture to provide a tool for fractionation of in vivo actives. Routine KB cell culture screening of synthetics has been resumed. Changes in in vivo screening protocols over the last year have further reduced the percentage of submitted synthetics not screened in vivo. The main objectives of routine KB screening of synthetics are to aid in the selection of highly cytotoxic but in vivo inactive agents for broader spectrum in vivo screening and for biochemical screening, and to provide a base of data for correlation of cell culture, in vivo, and biochemical activity. The in vitro screening program is being reviewed relative to overall program objectives. We need to continue use of cell culture screening for natural product bioassay and the KB system has been satisfactory for this purpose. However, other systems are being investigated for their applicability to this and other objectives. One study showed that a high percentage of KB actives were active against P388 in vivo. This raises questions regarding the qualitative and quantitative relationships among drugs with respect to activity against KB, P388, and L1210 cells in culture. Does the quantitative relationship of P388 activity to L1210 activity in vivo carry over to cell culture? A preliminary study of a few natural products suggested that such was the case. Would the use of P388 for cell culture screening of natural products uncover more cytotoxic extracts which would show P388 or L1210 activity in vivo upon partial or complete purification? The objectives of in vitro screening of synthetics also raises the question of the suitability of L1210 or P388 cells as an alternative to KB cells. Arguments in favor of developing economical L1210 and/or P388 in vitro screens are (a) ability to correlate cell culture activity with in vivo specificity against the same tumor, (b) ability to transplant cells from culture into animals with no change in growth characteristics or malignancy; already shown for L1210, and (c) ability to carry drug-resistant variants in culture for rapid studies of cross-resistance among analogs and the associated ability to confirm sensitivity or resistance against the same tumor in vivo. Arguments against development of alternative in vitro screens have been (a) current lack of data suggesting that they will pick up more or different cytotoxic materials, (b) our 15 years experience with KB, (c) the human origin of KB cells. Basic and development studies related to these questions are continuing at ADL. It would appear that either P388 or L1210 in culture has the potential to provide a suitable bioassay for natural product fractionation. Sensitivity of either compared to the KB assay is significant but selective. We are planning a larger scale study of these questions. Particular emphasis will be placed on assaying more crude extracts for further comparison of in vivo results with those for KB and L1210 or P388 in culture.

With respect to the use of cell culture assays to provide candidate drugs for more extensive in vivo screening, biochemical screening, the selection of promising drug combinations, and as an aid to studies of drug mechanisms, the use of cultures of readily re-implantable in vivo tumors and their drug-resistant sublines seems advantageous. While it is not clear the mechanisms of drug resistance in experimental models are duplicated in the clinical situation, the existence of a battery of specific drug resistant tumor variants in vitro and in vivo does provide a rational means for determining similarities or contrasts among structural analogs with respect to biochemical sites of action. Also, such information has been used to aid in the selection

of potentially synergistic drug combinations.

Biochemical screening, studies of biochemical mechanisms, and studies related to physiological distribution of drugs, were continued (see current Organizational Report, Biochemistry Section and Project Report, Serial No. 3576) at Yale University (67-1193), ADL (65-61), SRI (66-29), Cornell University (69-60) and MAI (68-1283). Two new promising folic acid congeners have been discovered: Baker's soluble antifol (NSC-139105) which unlike MTX (NSC-740) enters cells by simple diffusion and may attain substantial cerebral spinal fluid levels; and 5-methyl-tetrahydrohomofolate (NSC-139490) which has shown in vivo activity against both L1210 and an MTX-resistant subline of L1210 with high dihydrofolic reductase activity. Studies of patterns of sensitivity or resistance to NSC-139490 among a spectrum of MTX-resistant tumor sublines and relationships of activity to dihydrofolate reductase activity are underway. Studies of nitrosoureas showed that therapeutic effectiveness in vivo tended to correlate with alkylating activity while cytotoxicity in vitro correlated with carbamoylation. Current evidence suggested an aldehyde as an active in vivo metabolite of cyclophosphamide, and attempts to synthesize the active intermediate are underway. The SRI group is continuing to examine the inhibition of biosynthesis or function of DNA by agents that pass DN-2A for which the biochemical sites of action are not known. In addition, this group is examining drugs of interest for uniqueness regardless of in vivo activity against a spectrum of H.Ep2 cells in vitro including parent cells and cells lacking AMP pyrophosphorylase, IMP-GMP pyrophosphorylase, and/or adenosine kinase. The SRI group also found that a number of in vivo active inhibitors of the metal requiring enzyme, ribonucleotide reductase, (thiosemicarbazones, guanazole, and hydroxyurea) are chelating agents. SKF-525A and Phenobarbital which inhibit and enhance liver microsomal enzyme activity, respectively, were used as tools to study the role of liver microsomes in the activation of inactivation of antitumor drugs. Studies at MAI showed that both SKF-525A and Phenobarbital influenced the biological half-life of cyclophosphamide.

During the past year, three small contracts were initiated to explore biochemical screening as an aid in selecting promising anticancer agents. An Ad Hoc Committee was formed to select new contractors in this area. In selecting three projects from a number proposed, attention was given to those which might uncover unusual exploitable biochemical sites of inhibition; specifically, interference with cellular energy production (Univ. of Penna. 71-2188), inhibition of cell membrane formation (Southern Research, 71-2189), and effects of properties of nucleic acids particularly DNA-DNA helices and DNA-RNA hybrids (Yale Univ., 71-2339). In addition, a contract (72-2043) was initiated with the Scripps Clinic to prepare and evaluate vitamin B12 analogs.

The SRI group continued to use labeling techniques to study in detail the kinetics of growth of experimental tumors of interest. Five and 14-day old SC-inoculated LL tumors showed cell cycle times (Tc) of 16-19 hrs. and an s-phase (Ts) of 8.5-9.0 hrs. The percent labeled mitotic wave of 14-day old tumors indicated no change in distribution of generation times when the tumor reaches this stage of growth. The calculated proliferative fraction decreased from 0.67 to 0.38 between day 5 (340 mg.) and day 8 (575 mg.), but further change in proliferative fraction from day 8 to day 14 (2700 mg.) was minimal

despite a coincidental increase in mass doubling time from 2.9 to 6.3 days. This is consistent with an increased cell loss with growth or a change in the ratio of cell proliferation to cell loss. Results indicated that Tc and Ts of day-21 metastatic lung lesions were comparable to the Tc and Ts of 5-14 day old primary tumor. The growth fraction of the primary tumor tended to be smaller than for the metastases. Nevertheless, the data suggest that a chemotherapeutic schedule effective against the primary tumor should be effective against the metastases. Current emphasis is being placed on the kinetics of metastases and regrowths at the site of implantation following surgical removal of the primary tumor.

The study of the kinetics of cellular proliferation and the effects of drugs thereon using in vitro assay methods was continued at SRI (71-2098 and 66-29), U. S. Atomic Energy Commission (71-61) and the Upjohn Co. (68-1023). The SRI group has used proliferating and non-proliferating L1210 cells for estimating the cell cycle stage specificity of drug action. The Upjohn group used an asynchronous DON cell system. The AEC group used Chinese hamster (CHO) cells with an unusually long G_1 phase. Results obtained by these groups were for the most part consistent; apparent differences having been explained on the basis of the specific techniques used. The AEC group in collaboration with colleagues not supported by contract 71-61 have developed the Los Alamos Flow Microfluorometer which can rapidly measure a population of suitably stained suspended cells with respect to the relative numbers in G_1, S, or G_2+M. Cells can be so measured when taken directly from the in vivo site provided a suspension of single cells can be obtained. Ascites tumor cells as well as cultured cells have been used to date. We intend to pursue solution of technical problems that at present have hindered such measurements on primary or secondary tumor nodules and critical normal tissues; e.g., normal gut and bone marrow. The AEC group feels that these problems can be overcome but this will require considerable collaboration with other contractors or intramural laboratories. All data so far is consistent with values predicted using alternative, more cumbersome, methods. Potentially, the AEC approach could have major implications to our program inasmuch as, conceptually, one could measure the kinetic state of tumor cells before therapy, after therapy using various treatment regimens and drug combinations, and after surgery. Such information could be useful for predicting subsequent useful therapies. The rapidity of measurement could be important for measuring the kinetic state of human tumors.

Studies of immunotherapy as an adjunct to chemotherapy have emphasized (a) the potential use of immunological reactions against tumor-associated antigens via active or adoptive immunotherapy as an approach to enhancing drug activity against experimental virus-induced tumors, (b) in vitro and in vivo investigations of cell and humoral mediated immune reactions implicated in the immunotherapy of rodent tumors, (c) immunosuppressive or enhancement effects on host defense mechanisms, and (d) utilization of spontaneous and induced viral tumors as models for developing antitumor and antiviral assays for chemotherapy and immunotherapy. These studies are conducted at MAI (68-1283) and in-house (Project Report, Serial No. NCI-3577). Since the death of Dr. John P. Glynn, who was Head of the IIRM Section, Dr. Abraham Goldin, Associate Chief for Laboratory Research, DR&D, has assumed responsibility for supervision of our immunotherapy program in collaboration with the Chief, DEB. In addition to the

studies conducted by the IIRM Section, we have recently instituted an in-house project (Serial No. NCI-3583) for the detailed investigation of the immunogenicity of neoplastic cells by interaction of their surfaces with macromolecules. The latter project conducted by Dr. M. Lippman is aimed at exploiting cell surface changes accompanying transformation, uncovering cell growth inhibitors that bind to transformed cells selectively, and increasing the immunogenicity of tumor cells. A second objective of the latter project is the development of rapid in vitro micro-tests suitable for providing maximum information relative to cytotoxicity with minimal amounts of drug.

The DEB program represents a major and important part of the overall Chemotherapy Program. Contract and intramural projects are aimed at the attainment of specific objectives of the Chemotherapy Program Linear and Supplementary Arrays. These in turn form a significant segment of the National Cancer Plan objectives and approaches. Ongoing projects will be continued, modified, and expanded where necessary, and new projects will be implemented toward attaining objectives. Project modifications will be closely integrated with continual re-evaluation of current approaches and development of new approaches.

TABLE I. ANALYSIS OF DEB MONITORED ACTIVITIES

(1) - National Cancer Plan - Objective. Approach*

(2) - Linear (L) or Supplementary (S) Array Activity+

FY-'72 Funding (thousands)
ST - By Array Activity
T - Total

Contract No. / Institution	(1)	(2)	ST	T	PURPOSE
A. Contracts sponsored by Drug Evaluation Branch					
71-61	6.2	L-2AX	10	19	Screening for cell cycle stage specificity. Cell kinetics. Related R & D.
US - AEC	6.2	L-2B2	9		
71-2182	6.2	L-1XX	69	460	In vivo bioassay & primary screening. Detailed drug evaluation. Related R & D.
Battelle	6.2	L-2AX	281		
Mem. Inst.	6.2	L-2B2	110		
69-60	6.2	S-1XX	40	40	Asparagine metabolism of tumors.
Cornell Univ. Med. Coll.					
69-2115	6.2	S-1XX	19	19	Asparaginase, glutaminase, related enzyme studies.
Delaware, Univ. of					
63-622	6.2	L-1XX	221	971	In vivo primary screening & bioassay. Detailed drug evaluation. Related R & D.
Hazleton	6.2	L-2AX	500		
Laboratories	6.2	L-2B2	250		

TABLE I. ANALYSIS OF DEB MONITORED ACTIVITIES (Cont'd.)

Contract No. Institution	(1)	(2)	ST	T	PURPOSE
68-1021	6.2	L-1XX	100	1439	In vivo bioassay & primary screening. Detailed drug evaluation. Related R & D. Virology. Animal disease diagnosis.
IIT Research Institute	6.2	L-2AX	1233		
	6.2	L-2B2	106		
Indian Cancer Research Centre	6.2	L-1XX	(PL-480)		Plant collection & fractionation. Organic synthesis. In vivo screening & bioassay.
	6.2	L-2AX			
71-2259	6.2	L-2AX	490	560	In vivo primary screening and detailed drug evaluation. Related R & D.
Mason Res. Inst.	6.2	L-2B2	70		
70-2225	6.2	L-1XX	56	139	In vitro screening & bioassay. Related R & D.
Miami, Univ. of	6.2	L-2AX	83		
71-2188 Pennsylvania, Univ. of	6.2	L-2AX	11	11	Biochemical screening.
72-2042	6.2	L-1XX	19	39	Preparation and testing of Vitamin B12 analogs.
Scripps Clinic & Res. Fdn.	6.2	L-2AX	20		
66-29	6.2	L-1XX	250	502	Biochemical and cellular mechanism. Cell kinetics.
Southern Res. Institute	6.2	L-2B2	252		
71-2189 Southern Res. Inst.	6.2	L-2AX	27	27	Biochemical screening.

TABLE I. ANALYSIS OF DEB MONITORED ACTIVITIES (Cont'd.)

Contract Institution	(1)	(2)	ST	T	PURPOSE
71-2098 Southern Research Institute	6.2 6.2 6.2 6.2	L-1XX L-2AX L-2B2 L-6X2	100 1225 693 25	2043	Primary screening and bioassay in vivo & in vitro. Detailed drug evaluation. Cell kinetics. Development of new assays. Related R & D.
66-494 WARF Institute	6.2 6.2 6.2	L-1XX L-2AX L-2B2	392 231 71	694	In vivo primary screening & bioassay. Detailed drug evaluation. Related R & D.
Weizmann Inst. of Sciences (Israel)	6.2	L-1XX	(PL-480)		Cellular effects of macromolecules.
67-1126 Wisconsin, Univ. of	6.2	L-1XX	16	16	Bioautography of cytotoxic plant materials.
67-1193 Yale Univ. Med. School	6.2 6.2	L-2AX L-4X2	10 5	15	In vitro screening systems. Clinical pharmacology.
71-2339 Yale Univ. Med. School	6.2	L-2AX	55	55	Biochemical screening.
B. Multifaceted Contracts Sponsored by Other DR&D Units (Major Drug Evaluation Related Activities Monitored by DEB Staff).					
69-35 Bristol-Myers Co.	6.2 6.2 6.2 6.2	L-1XX L-2AX L-2B2 L-3X2	600 70 10 37	717	Antibiotic fermentation & isolation. In vivo & in vitro primary screening and bioassay. Detailed drug evaluation. Plant fractionation. Drug formulation production.

TABLE I. ANALYSIS OF DEB MONITORED ACTIVITIES (Cont'd.)

Contract Institution	(1)	(2)	ST	T	PURPOSE
Central Drug Res. Inst. (India)	6.2 6.2	L-1XX L-2AX		(PL-480)	Organic synthesis. Plant fractionation. In vivo screening.
65-61 Little, A. D Inc.	6.2 6.2 6.2 6.1 6.1 6.1	L-1XX L-2AX L-2B2 L-3X1 L-4X2 L-6X2	50 800 400 300 700 175	2425	In vivo & in vitro primary screening. In vitro bioassay. Detailed drug evaluation. Development of new assays. Cell kinetics. Experimental therapeutics. Viral oncology. Cryobiology. Related R & D.
68-1283 Microbiological Assoc. Inc.	6.2 6.2 6.2 6.2 6.1 6.1	L-1XX L-2AX L-2B2 L-2B3 L-3X1 L-4X2	600 650 450 30 150 330	2210	Detailed drug evaluation in vivo. Primary screening in vivo. Development of new methods. Experimental therapeutics. Drug distribution. Animal genetics. Related R & D.
69-2019 Research Triangle Inst.	6.2	L-1XX	140	140	Plant and other natural product collection & isolation. In vitro bioassay.
68-1023 Upjohn Co.	6.2 6.2 6.2	L-1XX L-2AX L-4X2	300 150 190	640	Antibiotic fermentation & isolation. In vitro screening & bioassay. Biochemical mechanisms. Experimental therapeutics. Related R & D.

TABLE I. ANALYSIS OF DEB MONITORED ACTIVITIES (Cont'd.)

Contract Institution	(1)	(2)	ST	T	PURPOSE
C. DEB Intramural Laboratory Projects (See Current Individual Project Reports)					
NCI Serial Number					
3576 Office of the Chief	6.2 6.2	L-2AX L-2B2		(Direct)	In vivo screening methodology. Detailed evaluation of new and old drugs. Biochemical mechanisms.
3583 Office of the Chief	6.4 6.2 6.2	S-1XX L-1XX L-2AX		(Direct)	Modifications in growth characteristics and immunogenicity of tumor cells. Effects of macromolecules on cell surface. Cell kinetics.
3577 IIRM Section	6.4 6.2 6.2 6.2	L-1XX L-1XX L-2AX L-2B2		(Direct)	Transplantation, serological, chemotherapeutic, & immunotherapeutic studies with virus induced murine tumors.
3578 Screening Section	6.2 6.2	L-2AX L-2B2		(Direct)	In vivo screening methodology. Detailed evaluation of drugs of specific clinical interest.

Footnotes, Table I

*Objective 6. To cure as many patients as possible and to maintain maximum control of cancerous process in patients not cured.

Approach 6.1. Develop and utilize methodologies of immunology, chemotherapy, surgery, and radiation (alone and in combinations) in the cure and palliation of cancer.

Approach 6.2. Determine new sources of chemical agents, new means of their selection, and additional screens for their use.

Approach 6.4. Enhance the host's ability to eliminate or prevent further development of cancer.

+L-Activities required to achieve goals as specifically outlined in Chemotherapy Program Linear Array. Research leading to development of improved methodologies for attainment of goals. Development of resources for attainment of goals.

S-Activities supplementary to Linear Array activities. Basic research of significance to the understanding of relevant scientific phenomena, which, if successfully elucidated, could have major impact on attainment of goals.

1XX - Acquisition of new agents. Studies of cellular control mechanisms.

2AX - Determination of anti-tumor activity of new agents. Development of improved assay methods.

2B2 - Detailed evaluation of new and old agents. Dose, route, & schedule dependency. Combination therapy. Cell kinetics.

2B3 - Initial animal pharmacology. Evaluation for absorption and antitumor activity after oral administration.

3X1 - Toxicology and pharmacology in large animals.

3X2 - Production and formulation for clinical trials.

4X2 - Clinical pharmacology. Initial pharmacologic studies in man associated with early clinical trials.

6X2 - Toxicological and pharmacologic studies in large animals and man associated with further development of a drug.

(Major DEB responsibilities associated with conduct of activities 1XX, 2AX, 2B2 and 2B3. Other activities are noted because they are conducted along with DEB activities in multifaceted contracts.)

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Serial No. NCI-3576
1. Chemotherapy, DR&D
2. Drug Evaluation Branch (DEB)
3. Office of the Chief
4. Microbiological Associates, Inc. (MAI)
Cancer Chemotherapy Department
Drug Evaluation Division
Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 - June 30, 1972

Project Title: Detailed Evaluation of Potential Antitumor Drugs.

Previous Serial Number: Same

Principal Investigator: John M. Venditti

Co-Principal Investigators: I. Kline, R. D. Costlow, R. J. Woodman, R. Cysyk, M. Gang, R. B. Field (MAI contract no. PH-43-68-1283).

Collaborating Units: Office of the Chief, DR&D (A. Goldin); Chemotherapy Research Division, MAI; Screening Section, DEB (B. J. Abbott); Experimental Therapeutics, C (J.A.R. Mead)

The Drug Evaluation Division, MAI, is a collaborative laboratory resource for the research staff, DEB, which acts in an advisory capacity in the planning, execution, and evaluation of tasks.

Man Years (NIH): Total 1. Professional 1.

Project Description: This is a multifaceted project devoted to the development and more efficacious usage of active antitumor drugs.

Objectives:

1. Detailed evaluation of new and old antitumor drugs with respect to treatment route and schedule dependency.

2. Conduct of studies leading to achieving therapeutic control of malignant disease in animals, emphasizing attempts to increase selective toxicity to tumor cells by modifying the way drugs are used individually and in combination.

3. Initial testing of synthetic and natural products in experimental in vivo systems appropriate for the identification of potential candidate drugs for clinical trial and confirmation of screening activity.

4. Development of new and improved assay systems arising from basic studies conducted in the Chemotherapy Research Division of this project and other DR&D-sponsored projects.

5. Conduct of studies related to biological "quality control" of experimental drug formulations.

Methods Employed:

Studies are conducted mainly in rodents with transplantable tumors (Leukemia L1210, drug-resistant variants of L1210, Lewis lung carcinoma) employing established procedures developed in the Drug Evaluation and Chemotherapy Research Divisions of this project and within DEB, DR&D.

Major Findings:

Only selected findings of special interest are summarized herein. Detailed descriptions of accomplishments are contained in the annual and interim progress reports, contract PH-43-68-1283.

Drugs discussed in this project report are listed below with their NSC numbers and the abbreviations used.

NSC No.	Name
740	Methotrexate (MTX)
755	6-Mercaptopurine (6-MP)
762	Nitrogen mustard (HN2)
1895	Guanazole
2760	Versene (EDTA)
8806	Melphalan
9848	Phenobarbital
10023E	Prednisone
23909	1-Methyl-1-nitrosourea
25154	1,4-Bis(3-bromopropionyl)-piperazine
26271	Cyclophosphamide (Cy)
27640	5-Fluorodeoxyuridine (5-FUdR)
32065	Hydroxyurea
33669	Emetine hydrochloride
39690	SKF-525A
45388	Imidazole-4-carboxamide,5-(3,3-dimethyl-1-triazeno)-(DIC)
52695	Phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)-,cyclohexylamine salt
56054	Pseudourea,2,2'-(9,10-anthrylenedimethylene)bis-2-thio-,2HCl
58514	Toyomycin (Chromomycin A3)
63878	Cytosine arabinoside, HCl (Ara-C)
66020	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-pyrrol-2-yl-
67574	Vincristine (VCR)
69856	Neocarzinostatin
71261	9H-Purine-6-thiol,2-amino-9-(2-deoxy-β-D-erythropentofuranosyl) monohydrate
71795	Ellipticine

NSC No.	Name
77213	Procarbazine
81850	Iproniazid
82151	Daunomycin
82196	Imidazole-4-carboxamide,5-[3,3-bis(2-chloroethyl)-1-triazeno]-(BIC)
83265	L-Alanine,3-(tritylthio)-
89473	Tetrahydrohomofolic acid
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-(MeCCNU)
100880	Camptothecin, sodium salt
102627	1-Propanol, 3,3'-iminodi-,dimethanesulfonate (ester), HCl
102722	Urea, 1,3-diallyl-
102816	5-Azacytidine
104800	1,6-Dibromodulcitol
107392	5H-Pyrazone, thiosemicarbazone (5-HP)
109723	1,3,2-Oxazaphosphorine,2-[bis(2-chloroethyl)amino]-3-(2-chloroethyl)tetrahydro-,2-oxide
109724	1,3,2-Oxaphosphorine,3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-,2-oxide (Isophosphamide,Is)
110435	Cesalin
111180	N-Acetylcysteine (NAC)
113089	Quinolinium, 6-amino-1-ethyl-4-[p-[[p-(4-pyridylamino)phenyl]carbamoyl]anilino]-,bromide,ethobromide
114347	Quinolinium,6-amino-4-[3-amino-4-[[p-(4-pyridylamino)phenyl]carbamoyl]anilino]-1-methyl-,p-toluenesulfonate, metho-p-toluenesulfonate
114900	Imipramine
118714	N-Acetyldaunomycin
119875	cis-Platinum (II)diamminodichloride (cis-Pt-II)
123127	Adriamycin
125066	Bleomycin
129220	O-2,2'-Cyclocytidine, monoacetate salt (Anhydro-Ara-C)
129942	2,6-Piperazinedione, 4,4'-ethylenedi- (ICRF-154)
129943	2,6-Piperazinedione, 4,4'-propylenedi- (ICRF-159)
135057	1,3-Bis(1-β-D-arabinofuranosyl-1,2-dihydro-]-oxo-4-pyrimidinyl)-2-thiourea
139490	5-Methyl-tetrahydro-homofolic acid
406021	9H-Purine-6-thiol, 9-β-D-arabinofuranosyl-
409962	Urea, 1,3-bis (2-chloroethyl)-1-nitroso-(BCNU)

Major Findings:

1. Combination Chemotherapy of Mouse Leukemia L1210.

Twenty-six drug combinations were tested against early ip or advanced sc-inoculated L1210 using a variety of treatment schedules. Those providing an increase in lifespan (ILS) greater than that achieved with the more effective of the individual drugs were cis-Pt-II + Ara-C or Emetine, Cyclophosphamide (Cy) + Camptothecin, Ara-C + 5-HP, DIC + Adriamycin, N-Acetylcysteine (NAC) + Isophosphamide (Is) and ICRF-159 + Daunomycin.

The previously reported therapeutic synergism of Ara-C + DIC was confirmed and extended to show the beneficial value of sequential therapy using MTX or BCNU following Ara-C + DIC. Thus, Ara-C + DIC followed by MTX or BCNU was more effective than continuation of Ara-C + DIC treatment. Moreover, recipients of tumors from donors which had been treated with Ara-C + DIC were (a) refractory to Ara-C treatment, (b) partially refractory to DIC, but (c) sensitive to MTX or BCNU.

A single NAC treatment protected normal and leukemic mice against the lethal toxicity of a single Isophosphamide (Is) treatment. By virtue of this protection, combinations of NAC + Is including a dose of Is which was lethal when used alone resulted in a degree of long-term survivorship among mice with L1210 which exceeded that achievable with the optimal dose of Is alone. In a typical experiment, the optimal dose of Is alone (300 mg/kg) increased the median survival time from 9.0 days (controls) to 23.5 days and produced 38% long-term survivors. At 500 mg/kg, Is alone was lethal to 100% of the mice. The combination of 1500 mg/kg NAC + 500 mg/kg Is resulted in no lethal toxicity and 100% of the leukemic mice survived for 50 days. NAC influence on Is toxicity and anti-L1210 activity was retained when NAC was given from one hour prior to 10 minutes after Is. In similar experiments, the influence of NAC on Cy toxicity and activity was not clearly established and these studies are continuing. Imipramine potentiated the toxicity of Cy in normal mice but failed to increase its therapeutic effect against early L1210. In mice with advanced L1210, Iproniazid, a monoamine oxidase inhibitor reduced the toxicity of Cy but the combination showed no therapeutic advantage over treatment with Cy alone. SKF-525A, an inhibitor of liver microsomal enzyme (LME) activity, and Phenobarbital, an enhancer of LME activity, prolonged and shortened, respectively, the duration of Cy action against L1210. While both SKF-525A and phenobarbital influenced the biological half life of Cy, in neither situation was the combination more effective than Cy alone in increasing the lifespan of the mice. NAC, Imipramine, Iproniazid, SKF-525A, and Phenobarbital were each ineffective against the tumor.

Both ICRF-159 and Versene (EDTA) decreased the lethality of a single Daunomycin treatment in normal mice. ICRF-159 was more effective in this respect than was EDTA. In leukemic mice, ICRF-159 + Daunomycin, each given every 4th day, was clearly superior to treatment with ICRF-159, the more effective of the individual drugs. Moreover, the optimal combination dosage consisted of ICRF-159 at its optimal dose when used alone plus Daunomycin at a dosage which was lethal when used alone (about twice the optimal Daunomycin alone dose).

2. Schedule Dependency of L1210 Leukemia.

Previous studies in this and other laboratories established the optimal schedule for Ara-C treatment of early (day 1) ip-inoculated L1210 to consist of treatments given every 3 hrs. on every 4th day. Against the early disease, this schedule typically produced more than 50% long-term survivors. Extension of schedule dependency studies of Ara-C showed that when treatment was delayed until day 3 the superiority of "around-the-clock" treatment was markedly reduced. When treatment initiation was delayed until day 5, the q3hr.,q4d. treatment provided no advantage over a single treatment given every 2nd or

4th day. The optimal dose per day, however, was 10-20 times greater when treatment was given in a single injection as compared with treatment in divided doses. Reports from other DR&D projects indicated that even when treatment is begun early, an increase in the total L1210 cell population (affected by increasing the tumor inoculum level) reduces the superiority of "around-the-clock" treatment over alternative schedules. A study was conducted to assess the influence of a reduction in tumor cell population by a non-cell-stage specific drug on the schedule dependency of subsequent Ara-C therapy. Mice with advanced L1210 were given an initial Cy treatment (Day 5) followed by Ara-C given either q3hr., q4d, or once every 2nd day from day 9. Both sequential combinations were markedly more effective than Cy alone treatment on day 5 or Ara-C alone given q3hr.,q4d. or once every 2nd day from day 5. Sequential therapy with Cy followed by Ara-C q3hr.,q4d. was somewhat more effective than sequential therapy with Cy followed by Ara-C once every 2nd day. However, none of the treatments produced long-term survivors among mice with advanced tumor.

N-Acetyl-daunomycin, an analog of daunomycin reported to produce less cardiotoxic effects than Daunomycin or Adriamycin was examined by the usual Type I schedule dependency protocol. No schedule dependency was observed, and overall, activity against L1210 was similar to that of Daunomycin and inferior to Adriamycin. The latter was schedule dependent for treatment q3hr. on day 1 only.

ICRF-159 and ICRF-154 were compared for activity and schedule dependency against L1210. ICRF-159 was most effective when given once every 4th day or q3hr.,q4d. ICRF-154 did not display schedule dependency and was inferior to ICRF-159.

O-2,2'-Cyclocytidine, monoacetate (Anhydro-Ara-C) was compared with Ara-C for schedule dependency against L1210 (in collaboration with Screening Section, DEB). Typically, Ara-C ip treatment produced a substantial number of long-term survivors when given q3hr.,q4d. to mice with early ip-inoculated tumor. Alternative schedules of Ara-C treatment, including treatment once each day, failed to elicit long-term survivors. In contrast, Anhydro-Ara-C, while as effective as Ara-C on the q3hr.,q4d. schedule, did produce a substantial number of long-term survivors when given once each day. The advantage of qd Anhydro-Ara-C treatment over qd Ara-C treatment was retained when the drugs were given orally. However, neither drug given orally produced long-term survivors on any schedule. Daily Anhydro-Ara-C was more effective than daily Ara-C against advanced SC-inoculated L1210 and against moderately advanced ic-inoculated L1210. A variant of L1210 developed for resistance to Ara-C was cross-resistant to Anhydro-Ara-C.

3. Other Studies of Activity Against SC-Inoculated L1210.

Twenty-nine agents which were active when given ip to mice with early ip-inoculated L1210 were examined for their effectiveness when given sc to mice with sc-inoculated L1210. At their optimal doses, 20 produced increases in lifespan $\geq$50%, seven produced an ILS of 30% - $<$50%, and only two failed to increase survival time by 30%.

ILS≧50%: NSC-1895, 45388, 66020, 71261, 77213, 83265, 100880, 102627, 102816, 104800, 107392, 109723, 109724, 113089, 114347, 119875, 129220, 129942, 129943, 406021.

ILS 30-49%: NSC-25154, 33669, 52695, 56054, 69856, 71795, 102722.

ILS<30%: NSC-58514, 123127.

4. Studies of Resistance and Cross-Resistance.

A Cy resistant subline of L1210 was developed. L1210/Cy was cross-resistant to Is, Melphalan, BIC, and BCNU but retained sensitivity to DIC, NSC-23909 (a nitrosourea lacking the bis-chloroethyl group), cis-Pt-II, and MTX. Discontinuance of Cy treatment of L1210/Cy carrier mice for eight generations did not nullify its resistance to Cy. However, the survival times of mice inoculated with L1210/Cy and untreated grew progressively longer with successive transplant generations. The latter phenomenon was also observed in the case of the DIC resistant variant, L1210/45388, in which drug induced changes in tumor cell antigenicity were identified.

5-Methyl-tetrahydro-homofolate (NSC-139490) retained its anti-L1210 activity against L1210/DF-8 which is resistant to MTX. Tetrahydro-homofolate (NSC-89473) had previously been shown to be more effective against L1210/DF-8 than against the parent line of L1210. NSC-139490 was more effective than NSC-89473 against both L1210 and L1210/DF-8 (in collaboration with J. A. R. Mead).

An Ara-C resistant subline of L1210, L1210/Ara-C, retained sensitivity to DIC, exhibited partial resistance to BIC and MTX, and was completely resistant to the Ara-C congeners, Anhydro-Ara-C and NSC-135057.

A subline of L1210 developed for resistance to both Ara-C and MTX, L1210/Ara-C/MTX retained sensitivity to DIC, 5-FUdR, 6-MP, BCNU, and BIC.

5. Studies with Lewis Lung Carcinoma (LL).

Because of the limited availability of C57Bl/6 inbred mice, the strain of origin of LL, BDF_1 hybrid mice were used for large-scale drug testing. Fifty-six experiments were evaluated with respect to relationship of tumor growth to survival of untreated mice and the reproducibility of response to Cy used as a positive control (20 mg/kg/day, days 1-11 and 180 mg/kg on day 1 only). On day 12 post im tumor implantation (brei), 68% of untreated controls exhibited tumors ranging from 500-2000 mg., 4% less than 500 mg., and 28% more than 2000 mg. Groups of mice with tumors in the 500-2000 mg. range exhibited median survival times between 22 and 30 days. There was an inverse relationship between mean tumor size on day 12 and median survival time. Results of Cy testing were sufficiently variable to indicate this drug as used to be a poor positive control. It is possible that the variability might have been due to the lack of dose response data or the inherent variability of the system with respect to response to drug treatment. If it were available in sufficient quantity, the investigation of Me-CCNU (single treatment) as a positive control would be appropriate. The growth and response to treatment in C57Bl/6 mice is currently under investigation.

Of the agents tested against LL, 15 produced tumor weight inhibition (TWI) of ≧60% and/or an increase in lifespan (ILS) ≧25%.

NSC No.	Schedule	ILS(%)	TWI(%)
95441	D1 only	81	88
	D5,12,19	104	100
409962	qd, 1-11	44	87
109724	qd, 1-11	100	100
	D1 only	50	100
	D5 only	64	92
26271	D1 only	50	100
	qd, D1-11	37	56
107392	qd, D1-11	58	100
125066	qd, D1-11	58	93
129943	qd, D1-11	55	72
25154	qd, D1-11	41	82
27640	qd, D1-11	29	89
32065	qd, D1-11	29	85
1895	qd, D1-11	28	32
10023E	qd, D1-11	<25	77
58514	qd, D1-11	<25	77
110435	qd, D1-11	<25	71
129220	qd, D1-11	<25	66

Four cell cycle stage specific agents (S-phase inhibitors) were tested for schedule dependency against early LL.

NSC No.	Schedule	ILS(%)	TWI(%)
107392	qd	70	100
	q4d	31	72
	q3hr.,q4d.	27	100
63878	qd	31	57
	q4d	46	86
	q3hr.,q4d.	20	50

NSC No.	Schedule	ILS (%)	TWI (%)
1895	qd	35	20
	q3hr.,q4d.	26	19
32065	qd	54	95
	q3hr.,q4d.	35	53

Although early (Day 1) LL has been estimated to exhibit a doubling time of about one day and a growth fraction of >70%, none of the agents were more active on an "around-the-clock" schedule than when given daily.

Using the Wexler India ink lung injection technique, ICRF-159 and 5-FUdR were studied for their ability to inhibit the appearance of metastatic tumor nodules in the lungs following im-LL implantation. Both agents retarded the appearance of lung nodules at sub-optimal (relative to ILS) doses which did not inhibit primary tumor growth.

One hundred eight new plant fractions were screened against LL. Nineteen either inhibited tumor growth by 60% or more or increased survival time by 25% or more.

VCR, Cy, Procarbazine, Prednisone, or HN2 followed by Bleomycin did not result in additive lethal toxicity in normal mice. Combination chemotherapy of LL with these agents is underway.

6. Primary Screening Production Report.

During calendar year 1971, the following numbers of computer processed screening tests were conducted.

Toxicity (normal animals)	585
L1210 Leukemia	4498
Lewis lung carcinoma	2762
P388 Leukemia	32
Total	7877

Proposed Course of Project.

Project proposes to continue the quantitative evaluation of drugs of potential clinical interest and the development of new methods for in vivo drug evaluation. Primary screening will continue at a level consistent with the needs of the Drug Evaluation Branch, DR&D. Emphasis will be given to tasks related to the NCI-Chemotherapy Program's convergence format and to research and development activities designed to supplement or complement these tasks. The development and use of drug resistant tumor sublines will be intensified to provide additional information for the evaluation of structural analogs and to provide leads for combination chemotherapy. Activities will continue to be carried out in collaboration with DR&D intramural laboratories.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

Objective

The objective of this section is to explore biochemical approaches to the improvement of drug therapy of human cancer. To accomplish this, work is underway in three areas; 1) studies with known drugs on what might be roughly termed mechanisms of action, but which embrace factors such as dose-timing, combinations of drugs, or any other aspect with a bearing on achieving greater anti-tumor specificity, 2) attempts to develop biochemical screens to identify drugs of interest because of specific sites of inhibition, and 3) production of new agents based on biochemical rationales. Some projects are being pursued through the continuation of contracts which have been in existance for a number of years and others are being undertaken with contracts initiated during the past year.

Accomplishments During the Past Year

1. Studies of Mechanisms of Action

A. Individual Agents

1. Folic Acid Antagonists (Yale University, PH-43-67-1193; and Arthur D. Little, PH-43-65-61). An outstanding contribution in this area was the selection of NSC-139105 (Soluble Baker's Antifol) for development to clinical trial. This was the culmination of the combined efforts the late Dr. B. R. Baker, a NCI grantee at the University of California, Santa Barbara, who synthesized the drug; Dr. Joseph Bertino, Yale University, whose work showed that the drug, unlike methotrexate (MTX), enters cells by simple diffusion and attains a substantial cerebral-spinal fluid level following i.v. administration to dogs; and Mr. Isidore Wodinsky, Arthur D. Little, who conducted in vivo testing of the many agents from which NSC-139105 was selected. It was evident very early from study of the death distributions during in vivo testing of the Baker agents that they had biological activity other than that of the usual antifol, such as MTX. Studies conducted in house showed that although lethal doses of MTX kill on about day three, toxic levels of the Baker drugs frequently resulted in deaths on day one. Accordingly, an experiment was designed to find to what extent the lethality of NSC-139105 could be reversed by citrovorum factor (CF). With single i.p. injections of NSC-139105 in 0.85% saline solution to weanling rats, 75 mg/kg was completely non-toxic, 150 mg/kg killed some of the rats, while 300 mg/kg was fatal to all. Deaths occurred within 2-5 minutes. The rats first trembled, then became prostrate and somewhat cyanotic. Prior to death respiration became shallow and quiet, but the heart beat was strong and not greatly accelerated. Upon autopsy, the rats were grossly normal; the heart

and great vessels were not abnormally filled with blood. Within 15-20 minutes those rats which survived began to move about, and within five minutes more were grooming themselves and eating. There was no weight loss and they remained normal thereafter. CF given ten minutes before NSC-139105 did not prevent the deaths. Since the cause of the acute deaths appeared to be sufficiently complex to require time and facilities unavailable here, Dr. Bertino was asked to examine the matter further.

2. Nitrosoureas (Southern Research Institute, PH-43-66-29). Agents of this class have two potential types of activity, alkylation and carbamoylation. An attempt was made to correlate these parameters with cytotoxicity in vitro and therapeutic effectiveness and toxicity in vivo. For twenty-two agents evaluated therapeutic effectiveness tended to correlate with alkylating activity and cytotoxicity with carbamoylation. The substituent group on N-3 tended to influence the rate of decomposition of the agent, and hence the rate of isocyanate formation, but had no effect on the reactivity of this group once it was produced. The hypothesis was proposed that these agents both alkylate DNA and prevent repair of the damage.

3. Active Principle of Cyclophosphamide (Southern Research Institute, PH-43-66-29). Evidence pointed to an aldehyde as the active intermediate of cyclophosphamide. Proof of this will be possible when the agent is synthesized under the Organic Chemistry Contract at this institution. Attempts to increase the life span of L1210-inoculated mice by swamping the liver aldehyde oxidase, which inactivates the active aldophosphamide, with other aldehydes was not fruitful. Of acetaldehyde, benzaldehyde, pyruvaldehyde, N-1-methylnicotinamide, menadione, and pyridoxal, only the last had activity, and that slight.

4. 6-N-Hydroxyadenosine (NSC-529410) (Southern Research Institute, PH-43-66-29). The primary mechanism of action of this agent remains unknown. Its use in vivo was followed by some inhibitions of incorporation of labeled precursors into DNA, but expected reversals, by addition of products of blocked steps, were not obtained. This agent is of interest, however, in that it is active against the 6MP-resistant L1210 (T/C% $\sim$ 200 compared to $\sim$ 160 in sensitive line). Some years ago the agent had the brief interest of Sloan-Kettering, but was found to be hemolytic.

5. 8-Azainosine (NSC-130279) (Southern Research Institute, PH-43-66-29) Continuing study of this agent has not revealed its primary site of action, but has eliminated most of the sites known to be inhibited by purine analogs, and has indicated that the active metabolite is probably 8-azainosine monophosphate. This agent too is active against 6MP-resistant L1210 (T/C% $\sim$ 160 in both resistant and sensitive lines).

6. Asparaginase (NSC-109229) (Cornell University College of Medicine, NIH-69-60). Study of the metabolic implications of the E. coli enzyme, and purification of enzyme from agouti serum have continued this year under the terminating contract with Cornell University College of

Medicine.

B. Other Biochemical Mechanisms (Southern Research Institute, contract PH-43-66-29).

1. Among the drugs acquired by DRD are many purine analogs. Unless these agents show in vivo activity significantly superior to established agents of this class or in a resistant line they are not developed further. To investigate these agents somewhat further for uniqueness, all acquisitions are to be tested by Southern Research Institute in a spectrum of H.Ep.#2 cells in vitro. In addition to the sensitive line, there are those lacking in AMP pyrophosphorylase, in IMP-GMP pyrophosphorylase, in adenosine kinase, in all three enzymes, and in all combinations of any two. A similar spectrum of cell lines for testing pyrimidine analogs is being developed.

2. The study of cell kinetics was in two parts: First, the effect of drugs on the progression of cells in culture through the cell cycle with findings, in brief, that thioguanine, hydroxyurea, cytosine arabinoside, guanazole, fluorodeoxyuridine, and fluorouracil prevented the transition from G1 to S, while 6-mercaptopurine and 6-methylmercaptopurine riboside allowed a very slow progression from G1 to S. Second, the T_c (16-19 hours) and T_s (8.5-9.0 hours) of primary Lewis lung tumor at 5-8 days and lung metastesis at 21 days (after primary implant) as well as the thymidine index did not differ significantly ($\sim$ 37%). The growth fraction decreased rapidly after days 5 to 8 in the primary tumor, and varied widely from one metastatic nodule to another.

3. In studies of ribonucleotide reductase, many inhibitors of this metal-requiring enzyme such as thiosemicarbazones, guanazole, and hydroxyurea were found to be chelating agents, an action which may in fact be their mechanism of inhibition. Desferal, a naturally occurring hydroxamic acid known as a strong chealtor of iron, inhibited the enzyme and enhanced the inhibition of DNA synthesis by the earlier mentioned agents.

Another agent, N-Hydroxyguanidine (NSC-44310) also inhibited incorporation of thymidine into DNA in vitro. While the agent closely resembles hydroxyurea in structure and both have about the same activity in vitro, hydroxygranidine is unlikely to be converted to hydroxyurea. It has different physical properties, such as basicity, so may, because of this, have a different physiological disposition and anticancer activity in vivo. The latter point is being investigated under SRI contract NIH-71-2098.

4. A search for inhibitors of DNA repair is planned. Because alkylated, or otherwise damaged, DNA can be repaired to some extent, agents which inhibit this action would be expected to potentiate the activity of alkylating agents. The in vitro system being developed will use ultraviolet light as a controllable source of damage, followed by determination of the effect of agents on the surviving fraction of

cells as shown by clone formation.

II. New Biochemical Screens

During the past year three small contracts were initiated to explore other approaches to the selection of promising anticancer agents, particularly agents which may act by unusual mechanisms.

1. Contract NIH-71-2188 with the University of Pennsylvania (Dr. Chance) was undertaken to find out whether it is possible to select useful anticancer agents whose primary mechanism of action is interference with cellular energy production. To accomplish this a study is being made of the correlation between in vivo anticancer activity, as determined by DEB protocol screening, and the effects of agents on mitochondrial energy coupling reactions and electron shuttle mechanisms of ascites tumor cells in vitro.

In an assay of thirty assorted agents, widely different effects have been found on respiration, uncoupling or inhibition of oxidative phosphorylation, and inhibition of electron transfer. A scan of more agents and the in vivo correlations are underway.

2. Contract NIH-71-2189 with Southern Research Institute (Dr. Hill) is devoted to selection of potential anticancer agents whose primary mechanism of action is inhibition of cell membrane formation. So far forty known agents of varying in vivo antitumor activity have been screened for their effect on incorporation of 1,2-C^{14}-choline and 1-C^{14}-D-glucosamine into total lipid (water insoluble materials) of Ca755 cells in culture. The contractor selected as a "level of interest" drugs which at ten times the ED_{50} concentration gave a ratio of ID_{50}/ED_{50} of under ten. On this basis ten of the agents were active inhibitors of choline incorporation; five of these ten inhibited utilization of glucosamine as well. Both groups of five contained in vivo active and inactive agents.

3. Contract NIH-71-2339 with Yale University School of Medicine (Dr. Canellakis) has as its objective assessment of members of several types of agents on a number of physical-chemical properties of nucleic acids or of agent-nucleic acid combinations. These may include changes in the spectrum of the agent, the nucleic acid or their combination, changes in the melting point of double stranded nucleic acid by the agent, the strength of binding, etc. Where the nucleic acid is DNA, the structural requirements for inhibition of DNA polymerases would emerge, which could lead to useful antitumor drugs with this mechanism of action, while the use of DNA-RNA hybrids would lead to useful agents against other polymerases. Work to date has been largely with the acridines. It has been demonstrated that the binding constant between DNA and this class of agents is subject to manipulation by structural changes in the acridine molecule. Thus the absolute structural requirements for acridine-DNA binding has become partially known, as have the permissible sites of variation and what groups increase or

decrease tightness of binding. Hence the groundwork is being laid for the production of, theoretically, the best possible acridine inhibitor of DNA replication. Work on other classes of agents and with other types of nucleic acids will follow the same pattern.

III. Production of New Agents

1. Contract NIH-72-2043 with Scripps Clinic and Research Foundation (Dr. Huennekens) was initiated to prepare and evaluate vitamin B_{12} analogs. This vitamin is known to be essential for cell division and maturation in man, and to be obtained entirely from exogenous sources. Hence if such antagonists, particularly of the active coenzyme form of the vitamin, have the ability to inhibit the replication of cells in culture or in host animals, they would be potentially useful agents for chemotherapy of cancer in man. By means of chemical and/or enzymatic syntheses a series of B_{12} analogs in which the upper axial ligand (above the Co-containing corrin ring as conventionally written) would be various nucleosides or alkyl groups, and the lower axial ligand would be a) the naturally-occurring base 5,6-dimethylbenzimidazole (colbalmin series) or b) water (cobinamide series). These analogs are to be tested: i) as inhibitors of replication of L1210 and/or other cells in culture, ii) as inhibitors of B_{12}-dependent enzyme systems, and iii) as inhibitors of the uptake of B_{12} by cells in culture. Promising agents will then be tested in L1210 leukemic mice.

While work has only recently been started a number of agents, all of the cobalmin series, have been prepared. Instead of adenosine in the upper ligand, there is arabinosyl adenine or N6-substutited adenosine, while in the alkyl series are four substituted methyl groups: F_3C, Cl_2FC, ClF_2C, and ethylamino. Elemental analysis and physical characterization are underway, with biological testing to follow. More agents with different substitutions will follow the same path.

2. Work in the study and production of biologically active polymers or of antitumor agents covalently attached to polymers is being done at the Weizmann Institute of Science, Rehovoth, Israel under PL-480 Agreement NIH-06-025-1. The objective of this work is either to develop new anticancer agents or to modify the effects of known agents to achieve greater utility, perhaps through changes in distribution or other effects.

Triornithine (NSC-152075) had been found in Israel to be taken up by E. coli cells and to block protein synthesis because of inactivation of ribosomes in such treated cells. Isolated E. coli ribosomes treated with triornithine were not inactivated when tested in a protein-synthesizing system made up of cell-free components.

When a supply of the agent was sent to DRD for evaluation, it was studied both in vivo and in vitro in mammalian systems. When tested in P388 leukemic mice by treating i.p. daily for nine days with 400

mg/kg/day there was neither toxicity nor increased life span. In KB testing in vitro, the maximum effect of about 20% kill was achieved at the top level of 100 μg/ml. Both of these observations are compatible with the concept of very little, if any, penetration of the drug into animal cells, as were the findings of Dr. Vincent Bono (Experimental Therapeutics) that triornithine at 75 μg/ml had in L1210 cells in culture no effect on either incorporation of tritiated thymidine into DNA or on labeled valine into total nuclear protein, the latter being an effect which would indicate that both RNA production and ribosome function were normal.

Seven melphalan derivatives in which either the amino or carboxyl or both groups of the phenylalamine part of the molecule were variously blocked were received (NSC-154852-154858) by DRD. The antitumor activity of these agents was compared to that of melphalan itself in P388 leukemia. At equimolar levels, only the derivative with the free amino group approached melphalan in activity. However this agent, in L1210 cells in culture, was also cytotoxic at a level not too far above melphalan, while all the others had considerably higher ED_{50} values. So the comparison in vivo is being repeated with doses extended upward to toxicity. The comparative effects on intracranial tumor are also under test.

IV. Plans for the Future

During the coming year enough data should accumulate to afford some preliminary judgment of the utility of the three new contracts started this year to broaden the selection process for new agents to those with mechanisms of action involved in cellular functions other than those directly related to nucleic acid biosynthesis.

There is still an unmet need for a greater capacity for identification of the mechanisms of action of the drugs in various stages of progression through the Decision Point Network.

Still needed as well are studies relating cellular membranes and function, and possible control of the latter by effects on the former. If the occasion permits, it might be of value to convene a committee of experts in the fields to consider the currently exploitable approaches in this area. These might well suggest practical approaches to influencing factors involved in emergence of a cell from G1 to S phase.

V. Publications

See current report, Office of the Chief, Drug Evaluation Branch.

13 rifamycin derivatives have been tested, with an *in vitro* assay, against Moloney Sarcoma Virus (MSV) and Moloney Leukemia Virus (MLV). Briefly, the assay involves growing mouse embryo fibroblasts in tissue culture, inoculating MSV or MLV and counting the cytopathic changes (foci or plaques) for a given titer of virus (with the MLV a source of defective MSV must be added, the XC cell). Drug is then tested by observing the highest non-toxic dosage (non-toxic to mouse embryo fibroblasts without virus) that reduces the virus titer. A 2-3 fold reduction in foci or plaques is considered significant. Table 1 lists the results of these studies.

Six of the rifamycin derivatives were tested for activity *in vivo* against viral-induced murine tumors. In the AKR lymphoma no significant activity was observed with the best results being a 30% ILS provided by N-demethyl rifampicin, NSC-143416 (Table 1). While most of the testing was against late stage disease some drugs were tested in AKR mice that were in remission (no palpable disease) following chemotherapy. Here too, no significant maintenance of remission-induction was observed as compared to placebo maintained controls. Rifamycin derivatives were also tested against primary MLV-induced leukemia (1^{o} MLV leukemia). Significant leukemic activity was observed for o-n-octyloxime -3-formyl rifamycin, NSC-143483 (62% ILS) and benzyl dimethyl rifampicin (BDR), NSC-143414 (157% ILS) (Table 1). Because of the marked activity of BDR in this study and the reports from other laboratories that this derivative is the most active rifampicin derivative against reverse transcriptase, further studies were performed and these are discussed in the next section. The *in vivo* experiment is being repeated.

To attempt to determine whether the drug was exerting anti-leukemic cell activity and/or anti-virus activity BALB/c mice diagnosed, day 0, with late stage 1^{o} MLV leukemia (palpable disease) were treated with the optimal dosage of BDR. As reported by M. Kende, A. I. Goldberg, T. Soares, and A. Goldin (*Bacteriology Proc.* in press) the following assays were done and the following observation made: (a) Virus levels in the sera were measured at various time intervals (day 0, 11, 18) and no reduction in circulating virus titer was seen compared to untreated controls. (b) The leukemogenic potency of the spleens of treated mice were assayed for by sacrificing animals at various intervals, inoculating their spleen cells into normal mice and sacrificing these mice 28 days later to determine the titer that produces 50% spleen enlargment, 32-fold reduction in spleen size was observed in mice that received spleens from drug treated animals as compared to mice that received spleens from untreated animals. (c) The virus titer of the spleens of treated mice was reduced 20-fold (day 11) and 5-fold (day 18) compared to spleens from placebo treated mice.

These studies suggest that BDR exerted a primary effect on the leukemic cell and not on the virus, because of: the reduced leukemogenic potency of treated animal's spleens, the reduced virus level in the treated animal's spleen presumably 2^{o} to reduction in leukemic cells and no change in the circulating virus titer.

To study further the activity of BDR, an experiment was designed utilizing MSV *in vivo*. Adult BALB/c were inoculated with MSV on day 0, and BDR was given from day -3 to +22. Percent tumor takes and tumor size were observed. There was

no inhibition of tumor takes but a definite decrease in tumor size. Because virus titer can be correlated with % tumor takes but not tumor size and cytotoxicity activity correlates better with inhibition of tumor size than with % inhibition of tumor takes this study also suggested that BDR was exerting an anti-tumor effect through a mechanism other than virus inhibition.

B. Interferon Inducers: Interferon is a low molecular weight protein that is synthesized by a host cell following viral infection and which then initiates a series of synthetic processes in nearby cells that ultimately prevents virus from infecting these cells. A number of non-viral interferon inducers have been utilized against both oncogenic and non-oncogenic viruses. Two of these agents were tested in this lab. Tilorone, as previously reported was inactive in vivo in the AKR system and had only minimal activity with MSV induced tumors in Balb/c mice.

Poly I:C, one of the most active interferon inducers, was tested against the MSV induced tumor in BALB/c mice and marked inhibition of tumor takes and tumor growth rate was reported by M. Kende and J. P. Glynn in Chemotherapy 16:281, 1971. It was also observed that by giving DEAE-dx, therapeutic efficacy was abrogated although interferon was still induced. This suggested that Poly I:C may exert anti-tumor activity through mechanisms other than interferon induction. Other workers have reported an immune enhancing effect with Poly I:C that may be responsible for anti-tumor activity. This was also studied in the MSV system and no enhancement of transplantation resistance was observed with Poly I:C.

C. Miscellaneous anti-viral agents: Two drugs have been reported to have activity against oncogenic and non-oncogenic viruses and were tested against MSV and MLV in vitro. Hydroxy guanidine, NSC-44310, at a dose non-toxic to normal cells inhibited MLV 4-fold, but less than 2-fold reduction in MSV-induced foci was observed. Adamson has reported 50% inhibition of MSV with in vitro assays. This drug will be studied further with in vivo studies of 1^{o} MLV Leukemia in BALB/c mice. 3-deazauridine, NSC-126,849, was reported to exhibit marked activity against some non-oncogenic viruses, throug an unknown mechanism of action. A 3-fold reduction in MLV-induced plaques was observed with in vitro assay but insignificant activity against MSV was seen This drug too will be tested in vivo.

IV. Chemotherapy of Murine Viral Induced Tumors

A. Spontaneous AKR Lymphoma: The spontaneous AKR Lymphoma has been used for chemotherapy screening in this laboratory over the past 3 years. Particul emphasis has been placed on: developing a two-stage therapeutic design, screening, for active anti-tumor agents, describing properties of the system relevant to diagnosis and attempting to link the disease to specific human neoplastic disorders.

(1) A. I. Goldberg, J. P. Glynn, M. Kende, N. Mantel, and A. Goldin (Cancer Res. 6/72) have described a 2-stage therapeutic design with a method for statistical analysis that takes into account all mice entering into a stud from day 0 including remitters and non-remitters. The objective was to presen a method of chemotherapy in a murine tumor system that would provide

analogies to clinic situations, particularly the remission-induction + remission-maintenance protocols used to treat acute leukemia in man. Actinomycin D has been shown to be effective in remission-maintenance. At 0.06 mg/kg (optimal dose), Q4D from day 7, Act. D produced a 40% ILS relative to placebo maintained controls. Methotrexate and 6-MP, both previously reported inactive for remission induction, have been retested at lower dosages. Southern Research has reported moderate activity against late stage AKR lymphoma with much lower doses than we previously had used. In retesting MTX against late stage disease we found 114% ILS using 0.75 mg/kg, daily for 15 days, and 57% ILS using 6 mg/kg, Q4D x 4 from day 1. In maintenance testing less than 10% ILS was observed. Several of the anti-viral agents have been tested in two-stage protocols, for remission-maintenance and were without effect. (See section on anti-viral agents).

(2) The AKR model appears to serve a useful function in drug screening. At the present time this laboratory is part of a co-ordinated committee, directed by Dr. Emil Frei, III, and in cooperation with Southern Research. The immediate goal of this Ad Hoc Committee is to test known clinically active agents in an attempt to relate the AKR system to specific human neoplastic disorders. Presently, the AKR tumor appears to combine features of human acute leukemia and human lymphoma (Hodgkin's and non-Hodgkin's) but is not consistently analogous to either condition. Vinblastine, an active drug against Hodgkin's disease, has now shown moderate prolongation of survival time on a daily treatment schedule. Further testing is in progress. The majority of experiments this year have involved testing rifamycin derivatives which are of interest because of their possible action in inhibitors reverse transcriptase and producing anti-tumor virus effects. Results are described in the section on anti-viral agents.

(3) Auxilliary studies have been undertaken that probe the natural history of the AKR lymphoma. As previously reported the WBC count does not correlate with either duration of survival or response to therapy. Diagnosis has therefore beeen based on the presence of palpably enlarged lymph nodes and spleen. To determine if any relationship existed between WBC count and size at diagnosis 100 mice with diagnosed disease were sacrificed following bleeding for WBC count. Organ weights (liver, spleen, thymus) showed no correlation with high or low WBC counts, further dissociating this parameter from state of disease. In progress is a study that involves performing clinical chemistry tests (liver functions studies, renal studies, electrocytes, Ca, P, proteins) on AKR mice either with late stage disease or in remission. This hopefully will give further information about the disease process and its correlation with specific human neoplasia.

B. Primary Moloney Leukemia Virus-Induced Leukemia (1^{o} MLV Leukemia): The 1^{o} MLV leukemia in BALB/c mice has continued to be used for testing anti-tumor drugs. The combination of cyclophosphamide and Palmo-Ara-C appeared moderately active, with 94% ILS. The remainder of the studies in this system involved screening rifamycin derivatives for potential anti-tumor activity. This virus-induced leukemia is particularly suitable for this type of study because circulating virus titer is readily measured and because we can correlate the in vivo observation with the in vitro assay that uses MLV. The details of the results are in Table 1 and the section on anti-viral agents. The number

of experiments involving 1° MLV leukemia in BALB/c mice has been reduced this year because we now use 30 mice per test group instead of 10, and because of the variability in the virus preparations which has necessitated altering some of the studies.

V. Antitumor and Enzymatic Activity of L-Asparaginase in Immune, Tolerant, and Normal Mice

L-asparaginase is an anti-tumor agent with a mechanism of action that depends on the tumor cell's need for an external supply of L-asparagine. The enzyme depletes the L-asparagine from the circulation and therefore interferes with the metabolism of the tumor cell. Resistance to the effects of L-asparaginase has been a major problem in the use of the drug and has been postulated to result from various processes including: induction of an enzyme system in the tumor cell capable of synthesizing L-asparagine, increased ability of the tumor cell to extract L-asparagine from exogeneous sources and the development of circulating antibody to L-asparaginase which forms a complex with the enzyme that is sequestered in the RE system. Whether this sequestered complex remains active is crucial to the argument favoring an immune basis for resistance. Our studies involve in vivo and in vitro (with D. A. Cooney, Experimental Therapeutics) experiments designed to investigate the immune basis for resistance to L-asparaginase.

A. In Vivo Studies: BALB/c mice were treated from birth or 8 weeks of age with weekly or biweekly injections of E.coli L-asparaginase. By testing the sera for Hemagglutination (HA) antibody to E.coli L-asparaginase it was observed that some mice developed significant antibody titers and some mice had negative antibody titer. The former group (HA of 1:8 ≥ were designated as Immune and the latter group (negative HA titers)) were designated as Tolerant. A third group of mice never previously exposed to L-asparaginase were called Normal. Two major in vivo studies were done with these mice, using P-1798 Lymphoma, known to be sensitive to L-asparaginase. Following challenge with P-1798, treatment with L-asparaginase produced clear anti-tumor activity in Tolerant and Normal groups, but Immune mice all died with tumor, without receiving any benefit from L-asparaginase. A second study compared Erwinia carotovora derived L-asparaginase with E.coli enzyme in these 3 groups of mice. The Erwinia carotovora enzyme, which does not cross-react with antibody produced with E.coli L-asparaginase, was equally active in immune, tolerant, and normal mice. The E.coli enzyme was not active in immune mice, but again effective in tolerant and normal mice.

B. In Vitro Studies (with Cooney, Exp. Therap.): Following a single injection of L-asparaginase, sera was taken at various time intervals from Immune, Tolerant, and Normal mice, and the levels of L-asparaginase and L-asparagine were measured. In Immune mice, 1/2 hour following injection, the L-asparaginase had been depleted and L-asparagine returned to pretreatment levels. Tolerant and Normal mice continued to have significant L-asparaginase detectable over 48 hrs. after injection and L-asparaginase was undetectable.

These studies suggest that immunity to L-asparaginase may result in sequestration of L-asparaginase and loss of anti-tumor and enzymatic activity. Also,

certain animals apparently become tolerant to enzyme following periodic injections while others become immune. Whether the direction of this immune response is controllable and whether animals found to be immune may be made tolerant are questions with major implications for future therapy with L-asparaginase.

Significance:

The purpose of these studies is to obtain a better understanding of the nature and biology of tumor associated antigens, the immunological response elicited in the tumor bearing host, the effect of chemotherapeutic agents on these responses, and the contribution immune phenomena might make to the overall therapeutic effect. The knowledge derived from such studies can be applied to model systems with transplantable and primary tumors in mice involving immuno-therapy and immuno-chemotherapy, and this may lay the framework for subsequent clinical studies. Also, studies involving the possible anti-viral properties of certain chemotherapeutic agents should contribute to the use of these drugs in the treatment of human neoplasia.

Proposed Course:

This section will continue a 3-pronged attack on murine viral-induced tumors. Immunotherapy studies will probe more efficacious methods of using active and adoptive regimens and more in depth work with assays to determine the kinetics of the immune response to tumor antigens. Chemotherapy will involve study with the AKR lymphoma and BALB/c primary lymphoma in terms of observing activity of known clinically active agents and searching for new active agents. Blood chemistry in AKR will be examined, also. Anti-viral agents will be evaluated in vivo and in vitro to screen for activity and to distinguish true anti-viral effects from cytotoxicity. Eventually it is hoped immuno-chemo and anti-viral therapy will be used in combination to achieve a highly effective antitumor regimen.

Publications:

See current Organization Report, Office of the Chief, Drug Evaluation Branch.

IN VITRO AND IN VIVO ANALYSIS OF THE ANTI-VIRAL ACTIVITY OF VARIOUS RIFAMPICIN DERIVATIVES

NSC#	NAME OF COMPOUND*	HIGHEST NON-TOXIC CONC. MCG/ML	IN VITRO ACTIVITY (FOLD REDUCTION) MSV	MSV SUPER-NATE	MLV	MLV SUPER-NATE	IN VIVO ACTIVITY (% INCREASE) (AT OPTIMAL DOSE) 1° MLV LYMPHOMA IN BALB/C	AKR SPONTANEOUS LYMPHOMA
113926	RIFAMPICIN	25	2	2	0	0	-	17
133100	RIFAMYCIN SV	2.5	5	8	-	2	-	7
143414	BENZYL DIMETHYL RIFAMPICIN	10	2	-	2	-	157	0
143416	N-DEMETHYL RIFAMPICIN	50	1.5	2	0	0	0	30
143446	DIPROPY HYDRAZONE RIFAMYCIN	10	3.7	10	0	-	-	-
143458	CARBOMETHOXY-DIMETHYL-PYRROLO-RIFAMYCIN	50	3.7	20	-	-	-	-
143471	O-DIPHENYLMETHYLOXIME RIFAMYCIN	5	0	10	3	-	-	-
143475	O-N-PENTYLOXIME RIFAMYCIN	5	0	6	0	-	-	-
143476	PHENYLPROPYLOXIME RIFAMYCIN	5	0	20	0	-	-	-
143481	DINITROPHENYL RIFAMYCIN	10	3-6	100	0	0	-	-
143483	O-N-OCTYLOXIME 3-FORMYL RIFAMYCIN	5	0	2	3.6	-	62	17
144126	DINITROPHENYL RIFAMYCIN	10	0	12	3.6	-	-	-
144128	DIPHENYL-HYDRAZONE RIFAMYCIN	10	0	12	2.5	-	-	-

TABLE 1 ACTIVITY OF 13 RIFAMYCIN DERIVATIVES AS MEASURED IN AN IN VITRO FOCUS COUNTING ASSAY OF MOLONEY SARCOMA VIRUS (MSV) AND MOLONEY LEUKEMIA VIRUS (MLV) AND AN IN VIVO TEST AGAINST LATE STAGE OF PRIMARY MLV VINDUCED DISEASE AND AKR SPONTANEOUS LYMPHOMA. SEE TEXT FOR DETAILS.

Serial No. NCI-3577
1. Chemotherapy, DRD
2. Drug Evaluation Branch
3. Immunotherapy and Immune Response Modifiers Section
4. Microbiological Associates, Inc. (MAI), Immunology Division, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Transplantation, Serological, Chemotherapeutic and Immuno-Chemotherapeutic Studies with Virus Induced Murine Leukemias and Sarcoma

Previous Serial No: Same

Principal Investigator: A. I. Goldberg

Other Investigators: A. Goldin (Principal Advisor), J. P. Glynn (Deceased, Former Principal Investigator), D. Houchens

Cooperating Units: Screening Section (B. J. Abbott), and Office of the Chief, Drug Evaluation Branch (J. M. Venditti), Experimental Therapeutics (D. A. Cooney, D. Young), NCI; MAI Contract PH-43-68-1283 (M. Kende)

Man Years (NIH):

Total: 3
Professional: 2
Other: 1
Plus animal care support from Mammalian Genetics and Animal Production Section, DRD.

Project Description:

Objectives:

1. To determine the efficacy of utilizing immunological reactions directed against tumor-associated antigens, through active immunotherapy or adoptive immunotherapy, in producing anti-tumor activity in murine viral-induced tumors.

2. To follow the cell-mediated and humoral immune reactions involved in the immunotherapy of murine tumor systems, with *in vitro* or *in vivo* assays.

3. To investigate the interactions between anti-tumor drugs and the host's immune defense.

4. To utilize spontaneous and induced primary murine leukemias as models for developing chemotherapy, immunotherapy and anti-viral regimens either singly or in combination and to develop assays to monitor the efficacy of these regimens.

Methods Employed: Standard Laboratory procedures were employed.

Major Findings:

I. Adoptive Immunotherapy

For a host to mount an immunologic reaction against a tumor cell requires the host to first recognize certain tumor antigens as foreign, and then to have the capacity to develop a cell-mediated reaction that will destroy the tumor cell. This type of reaction is analogous to transplantation rejection and the tumor antigens are thus referred to as Tumor Specific Transplantation Antigens (TSTA). In the cancer patient, either because of the disease process itself, or because of the use of drugs with immunosuppressive properties, the ability to effect an in vivo response against the tumor cell is markedly diminished. Adoptive immunotherapy, (allogeneic or syngeneic sensitized lymphocytes grafted into a host with tumor), is one method of bypassing the host's depressed immune system and achieving an immunotherapeutic effect. Our studies with murine viral-induced tumors involve adoptive immunotherapy in combination with chemotherapy. Graft-vs-host disease (GVHD), one of the main problems in the use of immunocompetent cell grafts, has also been studied.

A. Treatment of Moloney Virus-Induced Leukemia: Successful immunochemotherapy against LSTRA, a transplantable Moloney virus-induced leukemia of BALB/c mice has been reported by J. P. Glynn and M. Kende in Cancer Res. 31:1383, 10/71. Cyclophosphamide 240 mg/kg plus spleen cells from DBA/2 mice that were sensitized to Moloney Sarcoma Virus (MSV) produced marked prolongation in median survival time and increase in the number of 60-70 day survivors compared to cyclophosphamide therapy without cells. Normal DBA/2 spleen cells and DBA/2 spleens from animals sensitized to normal BALB/c tissue were without effect. Subsequent studies revealed that the DBA/2 anti-MSV spleen cells reacted specifically to MLV-induced transplantation antigens. No GVHD was observed with these allogeneic spleen cells presumably because DBA/2 and BALB/c share the major H-2 histocompatibility antigens and only differ at the minor loci. Spleen cells from DBA/2 mice immunized against BALB/c tissue produced early deaths in BALB/c mice, probably from GVHD. Of further interest were the late deaths in animals presumably cured of the LSTRA tumor with DBA/2 anti-MSV spleens. Virologic studies indicated that sarcoma and/or leukemia may have been induced by the grafted DBA/2 cells sensitized to MSV, and possibly may have carried defective MSV or MLV into the recipients. Because the use of fully competent MSV to sensitize the DBA/2 mice may have been responsible for late deaths in BALB/c mice cured of the LSTRA tumor, a different approach was utilized. A line of 3T3 mouse fibroblasts from Swiss, NIH mouse, that

contain MSV genome but not MLV (S^+L^-) has been established in tissue culture by Dr. R. Bassin, NCI, Viral Leukemia and Lymphoma Branch. In vitro assays of this tissue culture cell line are consistently negative for virus and therefore it was postulated that these cells may provoke immunogenecity to the TSTA without elaboration of sarcoma or leukemia virus. CBF_1 mice given two doses of 6.0×10^6 S^+L^- tissue culture cells 2 weeks apart exhibited moderate transplantation resistance to LSTRA (60% alive and tumor free 185 days after challenge with 10^5 LSTRA cells). Adoptive immunotherapy experiments are planned wherein donor mice will be sensitized to S^+L^- cells, and grafted.

B. Treatment of Spontaneous AKR Lymphoma: Following diagnosis of spontaneous lymphoma on day 0, AKR mice were treated with vincristine sulfate and prednisone on days 1 and 5 and re-examined on day 6. Mice in remission were placed on adoptive immunotherapy regimens, beginning on day 7, involving i.v. injections of allogeneic spleen cells taken from CBA/J mice. Donor CBA/J mice were sensitized to either AKR spleen cells or $E_{\delta}G2$, a Gross-virus induced tumor in C57Bl/6 mice. Mice alive 14-21 days after the allograft were killed and histopathlogic sections were made. Dr. David Young, Toxicology Branch, examined the sections and reported lymphoid atrophy, with reduction in lymphocytes and follicles, and eosinophilic fibrinoid material in the cortical regions of the lymph nodes. Most of the mice were completely free of lymphosarcoma. These findings suggested that the cause of death was GVHD, and also that a marked anti-tumor effect was achieved. Further studies therefore, involved attempts to reduce or prevent the GVHD (see next section), and are to be published in Proc. Amer. Assoc. Canc. Res., 4/72 co-authored by A. I. Goldberg and J. P. Glynn.

Early results of tests to protect AKR mice from challenge with transplantable AKR lymphoma have shown marked increase of MST in groups immunized either one or two times with E_{δ} Gross tumor cells. When spleens or sera from these mice were used in the ^{51}Cr release cytotoxicity test, maximum reactions were obtained at 10 and 17 days respectively. These in vitro results coincided with the time of greatest tumor resistance seen in the protection studies. Further studies will be carried out in an attempt to increase the immune response by use of active enhancement (e.g. BCG).

C. Studies on Treatment of Graft-vs-Host Disease (GVHD): In the clinic, therapeutic use of immunocompetent cells (e.g. Bone Marrow Transplantation) has been severly limited by the GVHD that subsequently occurs. While tissue typing and immunosuppressive drugs may occasionally decrease GVHD the problem remains a major stumbling block to this therapeutic modality. Also, Boranic, has shown that transient GVHD may be beneficial in terms of providing an anti-leukemic effect. Previous work in this laboratory demonstrated marked GVHD following C57Bl/6 spleen cell grafts into BALB/c mice. By giving anti-sera directed against the C57Bl/6 graft it was possible to break the graft and rescue the host. However, the duration of time that the graft was allowed to remain in the host was critical in terms of achieving an anti-leukemic effect. Mice with tumor, treated with allogeneic cells would die of GVHD if the graft persisted for over 4 days and would die of leukemia if the graft was broken with anti-sera 2 days after implantation. Several other methods of treating or preventing GVHD have been studied this year.

Antisera directed against the recipient's histocompatibility antigens were incubated with the immunizing tumor cells in attempt to produce donor cell that were tolerant of the recipient's H-2 antigens but sensitized to tumor antigens. An initial study using C57Bl/6 donors that were immunized to LSTRA that had been incubated with C57Bl/6 anti-Balb/c sera produced an inc in survival time compared to C57Bl/6 anti-LSTRA (without anti-sera) when given to CBF_1 mice with LSTRA. In AKR mice, a similar experiment was done using donor CBA/J spleen cells sensitized to $E_{\sigma}G2$ or AKR in the presence of CBA/J anti-AKR sera. A 3 day increase in survival time was observed compared to CBA/J anti $E_{\sigma}G2$ or anti-AKR without anti-sera.

Low doses of drugs such as methotrexate and cyclophosphamide have been used clinically to treat GVHD. We attempted to use these agents in the murine tumor systems. AKR mice immunosuppressed with cyclophosphamide and treated with CBA/J spleen cells sensitized to either AKR or $E_{\sigma}G2$ cells died with acute GVHD (with D. Young). Three experiments were performed using methotrexate 3-7 days after grafting sensitized CBA/J spleen cells into AKR mice that were in remission from spontaneous lymphoma. Slight prolongation in survival occurred. It appeared that if GVHD was prevented, animals succumb to leukemia but animals dying with GVHD were tumor free as suggested by spleen weight at death. Cyclophosphamide also is being evaluated in this system as a possible inhibitor of GVHD and initial studies indicate some degree of protection from GVHD may be possible with this drug.

II. Immune Response Modifers

A. Immune suppression: Most drugs active in the treatment of cancer also cause suppression of the patient's immune system. Studies with murin viral-induced tumors involve the relationship of immunosuppression to tumor growth and whether this suppression may be prevented with immune adjuvants.

The Moloney Sarcoma Virus (MSV)-BALB/c system has been used in these studie Adult mice, which were given only MSV, developed tumor which ultimately regressed in 80-90% of the animals. On the other hand, mice given cyclophosphamide one day prior to MSV ultimately died with disseminated tumor. The effect of the cyclophosphamide-induced suppression of the immune respon was observed indirectly by daily measurement of the local tumor. Cyclophosphamide's effect on humoral antibody was followed by injecting mice wit sheep erythrocytes (SRBC) on the same day as virus injection, bleeding the animals at various intervals (4, 7, 14 days) and determining the hemaggluti tion (HA) titer to SRBC. Mice receiving high doses of the drug (210 mg/kg) had marked reduction of titer to SRBC at days 4 and 7 with a return to norm at day 14. Lower doses of drug produced less suppression of the HA titer.

An *in vitro* ^{51}Cr release cytotoxicity assay is being employed to study the cellular and humoral immune respone of BALB/c mice given MSV. Results of such studies will aid in relating the kinetics of cellular and humoral immune response to the kinetics of tumor growth.

B. Immune enhancement: Non-specific active of immunotherapy of tumor with Bacillus Calmette-Guerin (BCG) has been reported by Weiss, Mathe and o Preliminary studies with L1210 and LSTRA were reported by our group in the

last annual report.

Recent experiments have been conducted to determine the effects of BCG on LSTRA and MSV in BALB/c mice. BCG inoculation was given 7, 14, 21, or 28 days before tumor or virus. When 10^5 LSTRA cells were inoculated, BCG had no effect. However, with a tumor cell dose of 10^2/animal 7 of 9 animals receiving BCG never developed tumors, while only 2 of 10 control animals were tumor free. When animals which received 10^5 cells were given cyclophosphamide (210 mg/kg) 7 days after tumor, the MST was increased to 20 days as compared to 10 days in the controls. By giving both BCG and cyclophosphamide the MST was increased to 22 days.

When BCG was administered to mice that later received MSV, tumors developed later, grew to a smaller size and regressed earlier then did those in untreated mice. If BCG (5 or 1 mg/mouse) inoculated mice were given cyclophosphamide 1 day prior to MSV, the tumors developed in 60-90% of the mice (compared to 100% of mice not given BCG), tumor development was delayed by 1-6 days and maximum tumor size was 2-4 mm smaller than in mice not given BCG.

These studies suggest that non-specific stimulation of the immune system with BCG increases the animal's defenses to tumor growth and reduces the immunosuppressive effects of cyclophosphamide.

Other studies are in progress using the methanol extracted residue of BCG (MER). Weiss has reported this non-viable bacterial product to be a better non-specific stimulant to the immune system than is BCG. MER was administered in a four week period to BALB/c mice. The mice were then given either 10^2 or 10^5 LSTRA cells and are being observed for reduced incidence of tumors and increased survival times.

III. Testing of Antiviral Agents

A review chapter has been written by A. I. Goldberg and A. Goldin entitled: "Current Trends in the Possible Use of Antiviral Agents in Acute Leukemia" that reviews the potential value that anti-viral agents may have in the treatment of neoplasia, as well as some of the evidence that relates viruses to neoplasia, etiologically. As discussed in this chapter, the inhibition of reverse transcriptase and the induction of interferon are 2 exciting approaches to therapy of virus-induced tumors and these modalities have been studied against murine virus-induced tumors.

A. Rifamycin Derivatives: Reverse transcriptase is an enzyme discovered in 1970 by Baltimore and Temin that is thought to have a critical role in the process of malignant transformation by RNA tumor viruses. It has also been found in leukemic cells from human acute leukemia patients, and has been postulated possibly to play a role in human leukemogenesis. Some of the rifamycin derivatives have been found to inhibit reverse transcriptase and this has led to studies in this laboratory that attempt to focus on 2 areas: (1) Screening various rifamycin derivatives for anti-viral activity, (2) Attempting to distinguish anti-viral activity from anti-tumor cell activity.

SCREENING SECTION

DRUG EVALUATION BRANCH

Objectives and Significance to the National Cancer Institute

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products in vivo and in vitro; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Chemotherapy Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coorination of work conducted by contractual arrangements with qualified institutions and through intramural laboratory studies. The present organizational report describes progress of the contract program. Major findings of the intramural laboratory program are summarized in current Project Report, Serial No. NCI-3578.

Activity against experimental tumors in vivo is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. In vitro activity provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for in vivo screening, for selecting compounds of potential interest in "slow-growing" tumor system models, and, for following fractions and isolation of active components of crude natural products with correlative in vivo activity, for providing information relative to mechanisms of drug action, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

A. General

The Screening Section is staffed by six senior professional and two junior professionals, one on detail from the Office of the Chief, DEB. Principal investigators are: B. J. Abbott (Head); D. Abraham (CO); M. C. Baratta (CO); R. I. Geran; N. H. Greenberg and M. M. Macdonald. Staff is supported by six technical and clinical personnel, two part-time biological aids and two statistical aids employed under work-study plans. Staff serves as Project Officers for five contracts devoted predominantly to in vivo screening; Battelle Memorial Institute (NIH-71-2182), Hazleton Laboratories (PH43-63-622), Illinois Institute of Technology (PH43-68-1021), Mason Research Institute (NIH-71-2259), and WARF Institute (PH43-66-494) and two contracts for in vitro screening methodology; University of Miami (NIH-70-2225) and University of Wisconsin (PH43-67-1126). In addition, staff, in collaboration

with the Chief, DEB; Chief, DDB; Associate Scientific Director and Chief, DR&D; and Scientific Director for Chemotherapy, monitored in vivo and in vitro screening, secondary drug evaluation, and methodolo tasks performed under multifaceted contracts with Southern Research Institute (NIH-71-2098), Arthur D. Little, Inc. (PH43-65-61), Bristol Laboratories (PH43-69-35), and Microbiological Associates, Inc. (PH43 68-1283). In reviewing biological effects of natural product fractio the Screening Section established directions of effort under addition DR&D contracts for natural product fractionation and isolation. Staf monitor the work of contract laboratories as indicated; coordinate to tal effort with other DR&D and Chemotherapy projects; design assays for selection of new active drugs, investigate usefulness and feasibility of new assays; recommend candidate drugs for clinical trial; and prepare data for publication and for review by the Drug Evaluatio Committee, Operating Committee, Experimental Therapeutics Area, Clini Trials Area, and for Chemotherapy Decision Network review.

B. Specific

In conduct of duties staff (1) review proposals for institution or re newal of contracts, recommend new project to Chief, DEB and prepare materials for Program and contract reviewing bodies; (2) provide guid ance and instruction to contract laboratories, monitor scientific and technical sufficiency, and indicate areas needing modification or clarification through frequent communication and project site visits; (3) establish priorities for testing and instruct laboratories to con duct non-routine experiments of special interest; (4) design experimental protocols for screening and detailed drug evaluation, define parameters of response, establish criteria for "activity"; (5) advise Program Analysis Branch (PRA), DR&D, of data retrieval needs and collaborate in establishment of procedures; (6) advise Mammalian Genetic and Animal Production Section, DR&D, of animal requirements; (7) organize, review, and summarize experimental results; (8) instruct labo atories regarding needed additional data; (9) advise natural product chemists under contract to Drug Development Branch of crude materials worthy of fractionation and isolation; (10) conduct studies related to the biological quality control of experimental clinical formulation (11) conduct studies leading to solution of drug evaluation related problems arising during toxicological study or early clinical trial o new drugs; (12) design and conduct basic research leading to the development of improved assays for screening.

Senior staff serve on the Drug Evaluation Committee (DEC), chaired by the Chief, DEB, which recommends to Chemotherapy Staff materials whi meet criteria for acceptance as clinical candidates. Four of the sta constitute the DEC's Data Review Subcommittee (DRS) which submits to full committee materials meeting minimal criteria for significant and reproducible activity. Staff collectively form the Screening Committ (chaired by the Section Head) which assigns materials that pass Decision Network 2 (DN-2) to contract laboratories for initial route and schedule dependency studies and for additional testing against a limi spectrum of signal rodent tumors; and summarizes data for considerat

at the Decision Network 2B and subsequent stages of the linear array. Thus, the Screening Section Staff represent a major part of an integrated program for the evaluation of more useful antitumor chemotherapy and the intensive study of new agents.

II. Significant Accomplishments

Accomplishments are summarized below in accordance with performance of specific tasks related to (A) determination of antitumor activity of new agents, (B) selection of candidate drugs for development toward clinical trial (C) detailed evaluation of new and old drugs of clinical interest, and (D) methodology, research and development.

A. Determination of Antitumor Activity of New Agents (Primary Screening)

Primary screening consists of the original testing of synthetics, plant, fermentation, and animal products submitted to the DR&D. Protocols for screening are designed to uncover initially a modest number of materials which are then subjected to progressively exacting requirements designed to select those with the greatest antitumor potential. During calendar year 1971, 10,151 new synthetics, 3015 new crude plant extracts, 1,215 new crude fermentation products, and 1,203 new crude animal products were submitted to the screening program. Table 1 shows the number of computer processible screening tests conducted for various types of materials in each assay system. Until August 1971, all materials were routinely screened in vivo against mouse leukemia L1210 utilizing two treatment schedules [every fourth day (Q4D) and daily treatment (QD 1-9)] to the extent permitted by the supply of material. Natural products were tested against mouse leukemia P388. Analysis of results of natural product screening to December, 1971 showed that active leads for fractionation and isolation emanating from the L1210 system for crude natural products were almost non-existent (Table 2). The decision was made to test natural products by the Q4D and QD 1-9 schedules in P388, testing only those found active in this system in the L1210 system. Increased testing capacity made it possible to intensify studies of other tumor models. B16 mouse melanoma was expanded to all screening laboratories with replicate studies designed to yield information necessary for statistical evaluation of the system as a potential screening tool. At the present time, this data is being analyzed. Expanded studies have also been carried out with the Lewis Lung mouse carcinoma (LL) and work has been intensified with first generation transplants AKR Leukemia. It is anticipated that the first generation AKR system will provide candidates for testing against spontaneous AKR Leukemia. Work has continued to a limited degree utilizing drug resistant tumors for secondary evaluation. In late 1970, the Screening Section was asked by Chemotherapy Program Staff to investigate the feasibility of increasing screening capacity to accommodate a doubling of the number of materials screened and the addition of two additional screening systems. A decision was reached to increase screening capacity from approximately 120,000 tests/year in FY 71 to a level of approximately 320,000 tests/year during FY 73. That latter level was based on our projection

that crude natural products would be screened against L1210, P388, and one additional system. In addition, it is anticipated that all synthetics except those received in an amount less than 65 mg. will be screened against L1210; 20% of all synthetics will be screened against a second tumor; and 10% against a third tumor. The projection for synthetics was based on a one year analysis of the amount received of each synthetic agent submitted to DR&D. We fully expect the screening level of effort to reach our 320,000 test/year goal by FY 73.

All primary screening contracts were competed during FY 71. Fifteen organizations submitted resumes and six submitted proposals. Five contracts were negotiated; three with institutions which were conducting screening at the time and two new contracts. The increase in screening capacity projected for FY 73 was attained by the addition of the two new contracts and by progressively increasing the level of effort in all primary screening laboratories.

In vitro (KB screening) continued against all new crude plant and animal products and fractionation products from crude plant extracts which had demonstrated coorelative in vivo activity. Developmental work has been initiated to supply the KB system with L1210 and P388 cells in culture. Synthetics have also been submitted for KB testing in order to elicit indications of biological activity for selection of candidates for testing in tumor systems under development.

B. Selection of Candidate Drugs Toward Clinical Trial

During calendar year 1971, 195 new synthetic materials passed the initial stages of screening and 462 materials were reviewed by DRS and/or DEC (Table 3). Seventeen were judged to have met current in vivo criteria for acceptance as clinical candidates and were referred to Chemotherapy Staff for DN-2 consideration. A total of 19 drugs passed DN-2. Table 4 lists the materials which passed DN-2 in calendar year 1971 and the experimental basis for the selection of each. Examination of the basis for selection of clinical candidates shows that 14 agents met DN-2A criteria in L1210. However, L1210 is a relatively insensitive tumor. Only 6 of 14 agents passing DN-2A in 1969 and 2 of 7 in 1970 passed on the basis of L1210 activity. The increase in L1210 actives in 1971 was attributed largely to changes in the L1210 protocol. The positive correlation between L1210 activity and clinical efficacy indicates that it should continue as our principal primary screen. However, institution of additional screening assays is of importance. Preferably, such assays should be different from those which have been widely used and terminated and should be logically based on experiment evidence that they might predict drugs of usefulness against slower growing tumors.

C. Detailed Evaluation of New and Old Drugs of Clinical Interest

The Screening Section is largely responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN-2 including

route and schedule dependecy tests, combination chemotherapy, studies of comparative effects against fast and slow-growing tumors, and other appropriate studies to determine the extent to which modifications in drug usage might increase therapeutic efficacy leading to cures of experimental tumors. The section reviews and summarizes such information for consideration of Chemotherapy Staff at Decision Points subsequent to initial acceptance. The section maintains the Selected Agents (SAC) File comprising comprehensive records of experimental results of the diverse assays, for active drugs and other agents of special interest.

1. Route and Schedule Dependency Studies

During calendar year 1971, 34 compounds were subjected to a total of 51 route and schedule dependency studies; 24 against L1210, 12 against P388, 3 against B16 melanoma, 6 against Lewis lung carcinoma, 5 against W256, and 1 against 5178Y. The Screening Committee reviewed and submitted to the Chief, DEB, summarized reports of such studies for 21 compounds. Initial route and schedule testing is designed to determine the effect on therapeutic effectiveness when the interval between treatment is varied. The usual Type I route and schedule dependency test involves IP treatment on Day 1 only, QD for nine days, every fourth day X 3, every eighth day X 2, every three hours on Day 1 only, every three hours q4D, and every three hours q8D. In addition, attention is given to the determination of activity via the SC route as indication of the effectiveness of parenteral treatment at a site different from that of tumor implantation, and via the oral route as an indication of absorption from GI tract. The IV route is included when feasible. In general route and schedule dependency tests are conducted using parallel groups of tumor-bearing and normal animals as an aid to estimating the degree to which lethal toxicity influences schedule dependency. It should be noted that the number of schedule dependency tests conducted exceeds what one might expect from the number of materials which passed DN-2. Usually for each drug, studies subsequent to the initial Type I test are required for confirmation of schedule dependency, determination of schedule dependency via various routes, and determination of schedule dependency of experimental clinical formulations. In addition, the selection of the clinical candidates from active members of a structural class has required comparative schedule dependency testing. Compounds with very extensive activity against the initially observed sensitive tumor may not appear to be schedule dependent because of the extreme sensitivity of the tumor, In such cases, schedule dependency tests are conducted against more advanced forms of the tumor and against other tumors with less inherent sensitivity to the compound.

2. Combination Chemotherapy

Based upon the emergence in recent years of a substantial data base pointing to the clinical effectiveness of drug combinations, there has been increased emphsis on the conduct of therapeutic trials in animals of new drug combinations which might lead to the development of more effective and curative therapy in the clinic. While DR&D has been engaged in combination chemotherapy for a number of years through its contract laboratories and since much of the design for combination therapy is developed within these laboratories, the lack of programs for the automatic processing of results had hampered staff monitoring of these investigations. In the past year, working in cooperation with the PRA, an automatic data processing system has been developed for reporting these results. The program has been tested on a trial basis and processing of this data has now become routine. The bulk of combination studies conducted during the past year have been carried out in our intramural laboratory (Project Report; Serial No. NCI-3578), at Microbiological Associates, Inc. (68-1283) and at the Southern Research Institute (71-2098). These laboratories collaborated in a concerted effort to initiate evaluation of all compounds in development toward clinical trial in limited combination studies. Related major findings are summarized in the reports cited above, in the Annual Progress Reports of the contract laboratories involved.

Review of combination chemotherapy studies indicated that the following drug pairs were more effective against L1210 than was the more effective individual drug.

Cytosine arabinoside (NSC-63878) + Picolinaldehyde thiosemicarbazone (NSC-729), 5-hydroxypicolinaldehyde-thiosemicarbazone (5-HP, NSC-107392), 6-Thioguanine (NSC-752), Guanazole (NSC-1895), 5-Fluorouracil (5-FU, NSC-19893), Cyclophosphamide (NSC-26271), Mitomycin C (NSC-26980), Hydroxyurea (NSC-32065), DIC (NSC-45388), BIC (NSC-82196), BCNU (NSC-409962), CCNU (NSC-79037), or ICRF-159 (NSC-129943).

Palmo-Ara-C (NSC-135962 + 6-Thioguanine (NSC-752)

Methotrexate (NSC-740) + Guanazole, Actinomycin D (NSC-3053), 5-FU, Cyclophosphamide, Camptothecin sodium salt (NSC-100880), or BCNU.

6-Mercaptopurine (NSC-755) + Daunomycin (NSC-82151) or BCNU.

DIC + Melphalan (NSC-8806), 5-FU, Cyclophosphamide, Daunomycin (NSC-82151), or Adriamycin (NSC-123127).

Cyclphosphamide + BIC, 5-HP, BCNU, or cis-pt-II (NSC-119875).

Cis-Pt-II + 5-FU, Emetine (NSC-33669), BIC, ICRF-159, or I-phosphamide (NSC-109724).

ICRF-159 + Daunomycin, Camptothecin sodium salt, or 5-Azacytidine (NSC-102816).

I-phosphamide + N-Acetylcysteine (NSC-111180).

3. Biological Quality Control of Experimental Clinical Formulations

As a support function, we are collaborating with the DDB, Clinical Drug Distribution Section, in comparing the in vivo activity of formulated drugs with the activity of the bulk compound. The screening system which signaled the activity of the bulk drug is used for this comparison. In addition, considerable effort has been expended in studying the toxicities of non-routine vehicles anticipated for clinical formulation. Such studies can be extensive inasmuch as the potential toxicity of a vehicle must be examined carefully, especially when intensive parenteral treatment schedules are indicated. In the past year, comparisons of experimental and final formulations with bulk material have been carried out for 15 drugs in development toward clinical trial and were reported to the Drug Distribution Section.

4. Further Evaluation of "Deferred" Compounds

Among those compounds maintained in the SAC file are a group which have demonstrated reproducible activity in vivo but lack sufficient activity to be considered as clinical candidates. Since many of these represent new classes of compounds, it is felt that they merit further study. For this reason, we are in the process of re-examining these "deferred" materials. In many cases, minimum but significant activities were observed using obsolete screening protocols. To the extent permitted by the amount of material available, approximately 760 compounds have been sent for P388 testing using the Chronic and Intermittent schedules. Additionally, greater utilization has been made of the B16 melanoma and Lewis lung carcinoma tumor systems. In collaboration with the Southern Research group (Contract NIH-71-2098), 173 agents which had been deferred on the basis of L1210 activity were re-examined as potential candidates for schedule dependency testing to determine whether the activity require for DN-2A passage might be achieved on a non-routine schedule. Of these, 28 were selected for schedule dependency studies on the basis of (1) chemical uniqueness relative to known anti-tumor drugs, (2) potentially interesting mechanisms of action, and (3) reproducibility of existing primary screening data. In the evaluation of deferred compounds, new data received is reviewed in accordance with current criteria and practice.

5. Other Studies

Additional studies of materials which are in early phase 1 clinical trial or regarded as the most likely candidates for early clinical trial included investigations related to the (a) influence of the tumor site on drug effectiveness (e.g., effects against intracranially inoculated tumors as an indication of the hindrance to therapy of the "blood-brain-barrier"), (b) effect of new therapy on the development of resistance or on overcoming resistance to treatment, and (c) mechanisms of drug action.

D. Methodology (Research and Development)

Research and development consists primarily of studies to establish experimental designs which may lead to improved methods of drug evaluation and to the selection of drugs with greater clinical potential. A major portion of this work is devoted to the establishment of experimental protocols for screening and the evaluation of additional test systems and their correlations with both the current test systems and with clinical effectiveness.

Efforts are continuing to be directed to the study of experimental tumors with relatively long doubling times. The search for a suitable model for screening, as a part of the continuing effort to uncover drugs of clinical usefulness against solid tumors, had been hampered in the past by the limited screening capacity available in the total program. However, attention has been given to the testing of a number of known active and inactive materials against the IM - LL tumor using both tumor diameter and survival time as parameters of response. Compounds under study have included selected synthetics and natural products previously found active in the old LL-tumor weight inhibition system. Schedule dependency studies have been carried out against early and advanced LL with drugs of special interest. For example, Bleomycin (NSC-125066), which was accepted for clinical trial in our program on the basis of Japanese reports of experimental and clinical efficacy, was found active against LL and was studied for route and schedule dependency in this system. Work is also continuing against the established SC - LL tumor and a review of its potential as a routine screening system is scheduled. The IP B16 Melanoma (B1) tumor was sent to all screening laboratories during this report period. Studies of innoculum levels, drug sensitivity and reproducibility are in process. A number of compounds of current interest and select other SAU compounds were submitted for testing in this system. A small number of agents were examined for schedule dependency against B16. It is noteworthy that SC-innoculated B16 appears less sensitive than the IP-innoculated tumor to IP treatment. However, the SC tumor does provide an additional parameter for evaluation, i.e., reduction in size of the primary tumor. For this reason, studies are continuing with this tumor at two laboratories and repeat drug sensitivity experimental designs are currently being conducted. The selection of a "slow growing" tumor as an adjunct to the basic L1210 screen will require duplicate testing of a number of materi-

als and the screening of a reasonable number of random acquisitions in order to establish a data base sufficient for analysis of responses and for establishing quantitative criteria for activity. In addition, selection must take into account the kinetics associated with tumor cell proliferation and extent of metastasis.

III. Related Projects

There have been many changes in the protocols for screening since their last publication in 1962. While these changes over the years have been transmitted to those associated with the DR&D program, the need to publish a comprehensive edition has been recognized for some time so that these procedures would be available to the scientific community in general. These revised protocols have now been submitted for publication. Protocols and assay systems will continue to change, but publication in the summer of 1972 of these revised protocols is expected to give us again a complete, fundamental article which will be sufficiently comprehensive to need a minimum number of revisions.

In order for the screening program to function properly, all contract laboratories must employ tumor lines with uniform biological characteristics and host animals of uniform high quality. To achieve this uniformity, the Screening Section maintains frozen tumor banks at specified laboratories and from these, new tumor lines are dispensed to all screening laboratories at specified intervals. In addition, the tumor lines are monitored by the Screening Section and if it appears that the characteristics of a tumor line in use may be changing, new tumor lines are requested and sent to all contract laboratories from the tumor bank.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section. Selected screening contract laboratories perform studies related to the diagnosis and control of animal disease and to provide the quality control necessary for maintenance of the quality of the animals utilized in the DR&D program.

The Screening Section, as the major user of the DR&D data processing system, continues to work closely with PRA by advising and assisting in the planning of modifications and the monitoring and review of data necessary to an ongoing data processing system. In addition, we continue to review for need, all requests for special queries to our data files.

IV. Proposed Course of Projects

The Screening Section will continue the supervision of testing and the evaluation of data for synthetic compounds, plant, animal and fermentation products which are submitted to the DR&D. Emphasis will continue to be placed upon several diverse but related projects of interest to the Chemotherapy Program of the NCI. Utilization of in-house laboratory facilities to provide rapid and accurate evaluation of both test systems and compounds of major program interest will continue. This will involve basic research in

areas related to cancer chemotherapy and pilot systems aimed at devising new and improved tumor assays prior to their establishment in contract laboratories. Effort will continue both in the intramural program and in the contract areas to devise, implement, and evaluate more sophisticated systems to uncover drugs of potential clinical usefulness. Development of a broader program for drug evaluation against "slow growing" tumor systems is underway.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

TABLE 1

Number of Computer Processed Screening Tests Conducted
Calendar Year, 1971

Test System	Synthetics	Plant Products	Fermentation1/ Products	Total
In Vivo				
Leukemia L1210	74,067	16,070	12,287	102,424
Leukemia P388	3,268	32,023	18,158	53,449
B16 Melanoma	1,386	33	4	1,423
Walker 256				
(Tumor Inhibition)	1,002	1,245	652	2,899
(Survival)	1,412	41	16	1,469
Lewis Lung				
(Tumor Inhibition)	1,285	18	4	1,307
(Survival)	1,379	113	4	1,496
Others	1,765		15	1,780
Toxicity Studies				
(Non-tumored Animals)	2,835			2,835
Total In Vivo	88,399	49,543	31,140	169,082
Cell Culture	3,332	8,620	533	12,485

1/ Includes 4,182 animal products

TABLE 2

Yield of L1210 Confirmed Actives (ILS ≧ 25%) Among Crude Plant Extracts Since Institution of P388 and L1210 Dual System (to December, 1971)

1.	No. tested	∿12,000
2.	No. confirming in L1210 and P388	1
3.	No. confirming in L1210 only	0
4.	No. confirming in P388 only (Some had been active in earlier screens; i.e., W256, S180, etc., but not L1210)	361
5.	No. of Gp. 4 confirming in L1210 during fractionation	28
6.	Pct. of materials selected as crudes on basis of P388 only activity that confirmed in L1210 during fractionation	28/361 = 7.8%
7.	Pct. L1210 actives from random screening in L1210 over the years (13/15 were active in P388 as fractions or crystalline materials. 2/15 have not been tested in P388. Since these were not screened in P388 as crudes, they are not included in 1-6 above).	15/49,290 = 0.03%

TABLE 3

Status of Active Compounds
Calendar Year, 1971

New actives	195
No. of compounds reviewed by DRS and DEC.	462
DRS.	345
DEC.	117
Recommended to Chief, DR&D as candidates for Chemotherapy Staff (DN-2) consideration as potential candidates for clinical trial	17
Passed DN-2	19
Route and schedule dependency studies (experiments) scheduled	51
No. of compounds reviewed by Screening Committee	21

TABLE 4

Compounds Passing Decision Network 2 During Calendar 1971

NSC	NAME	BASIS	DATE
12169 E	Estra-1,3,5(10)-triene-3,16α,-17β-triol	Endocrinology Program	1/6/71
	Estriol		
74437	Piperazine,1,4-bis(2,3-epoxypropyl)-	L1210	6/28/71
96349	Coralyne	P388	11/1/71
	Dibenzo[a,g]quinolizinium, 2,3,10,11-tetramethoxy-8-methyl--chloride		
126849	2,4(1H,3H)-Pyridinedione, 1-β-D-ribofuranosyl-	L1210	11/1/71
129220	6H-Furo[2',3':4,5]oxazolo-[3,2-a]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-,monoacetate (salt)	L1210	11/1/71
	O2,2'-Cyclocytidine,monoacetate (salt)		
132313	Galactitol,1,2:5,6-dianhydro	L1210	5/17/71
133100	2,7-(Epoxypentadeca[1,11,13]-trienimino)naphtho[2,1-b]furan-1,11(2H)-dione,5,6,9,-17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-, 21-acetate	Inhibition of "reverse transcriptase"	5/17/71
	Rifamycin SV		
135962	Cytosine,1-β-D-arabinofuranosyl-, 5'-palmitate	L1210	5/17/71
	Palmo-ara-C		

NSC	NAME	BASIS	DATE
139105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenoxy]-N,N-dimethyl-m-toluamide (1:)	WA256 (im)	5/17/71
141537	Spiro[2,5-methano-1-benzoxepin-10,2'-oxirane]-5a(6H)-methanol,2,3,4,5,7.9a-hexahydro-3,4-dihydroxy-5,8-dimethyl-, 4,5a-diacetate	L1210/P388	6/28/71
D141540	Epipodophyllotoxin,4'-demethyl-, 4,6-O-ethylidene-β-D-glucopyranoside	L1210	5/17/71
141549	Methanesulfon-m-anisidide, 4'-(9-acridinylamino)-,monohydrochloride	L1210	5/17/71
143647	Propionic acid,2-amino-3-(hydroxynitrosamino)-,DL- Alanosine, DL-	L1210	11/1/71
143769	Methanol extraction residue (MER) of BCG	L1210	1/6/71
143969	Fluoren-9-one,2,7-bis[2-(diethylamino)ethoxy]-,dihydrochloride Tilorone	Reticulum cell sarcoma	2/8/71
145668	6H-Furo[2',3':4,5]oxazolo-[3,2-a]pyrimidine-2-methanol, 2,3,3a,9a-tetrahydro-3-hydroxy-6-imino-,monohydrochloride O2,2'-Cyclocytidine, monohydrochloride	L1210	11/1/71
145669	4(1H)-Quinazolinone, 2,3-dihydro-2-(1-naphthyl)-	L1210/P388	8/16/71

Table 4 cont'd.
Page 3

NSC	NAME	BASIS	DATE
146396	[1,3]Benzodioxolo[5,6-c]phenanthridine, 12,13-dihydro-2,-3,13-trimethoxy-12-methyl- Nitidine, 12,13-dihydro-13-methoxy-	L1210/P388	8/16/71
146397	Nitidine, chloride, dihydrate [1,3]Benzodioxolo[5,6-c]phenanthridinium,2,3-dimethoxy-12-methyl-, chloride, dihydrate	L1210/P388	8/16/71

Serial No. NCI-3578
1. Chemotherapy, DR&D
2. Drug Evaluation Branch
3. Screening Section
4. Building 37

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Basic Studies for Drug Evaluation Utilizing Antitumor Screening

Previous Serial Number: Same

Principal Investigator: B. J. Abbott

Co-Principal Investigators: D. Abraham, M. Baratta, R. Geran, E. Wagshal

Cooperating Units: Immunotherapy and Immune Response Modifiers Section (A. Goldberg); Drug Development Branch (R. Engle), (P. Davignon); Illinois Institute of Technology Research Institute (Contract 68-1021, A. Shefner); Microbiological Associates, Inc. (Contract 68-1283, I. Kline); Experimental Therapeutics (J. A. R. Mead).

Man Years: Total : 4 1/2

Professional: 1 1/2

Other : 3 (includes only 1 full-time person, other staffing with part-time employees)

Project Description and Objectives

The Screening Section intramural laboratory conducts basic studies related to in vivo antitumor screening emphasizing the development of new and improved methods, and the detailed evaluation of drugs of interest to the Chemotherapy Program.

Methods Employed

Studies were conducted mainly in mice using transplantable leukemias L1210 and P388, melanoma B16, and Lewis Lung carcinoma; and in first generation transplanted AKR leukemia. Established methods and new procedures developed within this project were used.

Major Findings

1. First transplant generation of AKR leukemia (with A. Shefner). Spontaneous development of leukemia in AKR mice has been attributed to the

presence of a vertically transmitted virus. Over 90% of AKR mice develop leukemia between 6-12 months of age. Progression of disease is rapid once palpable spleens and lymph nodes are noted; death usually occurring within 2-3 weeks. The virus induced disease can be transmitted to young AKR mice by implantation of leukemic splenic or thymic tissue. Schabel et al. (Southern Research Institute) have determined the growth characteristics of this first passage of the leukemia. The first passage disease mimics the spontaneous disease in that the median survival time following onset of splenomegaly is similar. Also, in the advanced state, first passage AKR leukemia exhibits growth kinetics approaching that of the spontaneous tumor. Long-passaged AKR leukemia exhibits growth characteristics similar to L1210. Difficulties inherent in screening drugs against the spontaneous disease, specifically, obtaining a sample population at a similar state of disease large enough for the testing of even a moderate number of drugs, are obviated in the first-passage system. Relatively few spontaneous tumor donors can yield an adequate and controlled first passage test population. During the past year we have made considerable progress in establishing a reporducible first passage AKR leukemia test system and in developing procedures that would be appropriate for relatively large-scale screening. Hopefully, such a system will provide a rationally based pre-screen for the selection of new agents for further evaluation against spontaneous AKR leukemia; either for induction or maintenance of remission. A tentative protocol for testing against first passage disease has been developed. Thirty-one drugs were tested in accordance with this protocol (13 at both NCI and IITRI, 12 at NCI only, and 6 at IITRI only) to determine activity and reproducibility between laboratories. For drugs tested in both laboratories, similar results were obtained although some differences in toxicity levels were observed. Drugs are being tested against early, moderately advanced, and advanced disease. Drugs showing initial activity (>25% ILS) as of 2/72 were:

NSC	Name	Treatment Begun Day 1	Day 5	Day 9
740	Methotrexate	+	NT	+
19893	5-Fluorouracil	+	NT	+
26271	Cyclophosphamide	+	NT	+
33669	Emetine	+	NT	+
45388	DIC	+	+	+
63878	Cytosine Arabinoside	+	+	+
71795	Ellipticine	+	NT	+
82196	BIC	+	NT	+
100880	Camptothecin, Na	+	NT	-
102627	"dimethane sulfonate"	+	+	-
102816	5-Azacytidine	+	NT	-
109724	I-phosphamide	+	NT	+
119875	cis-Pt-II	+	NT	-
135758	"Actinomycete P"	+	+	-

2. Structure-Activity Relationships of Analogues of Cytosine Arabinoside (with I. Kline and J. A. R. Mead).

As part of an overall program study of Ara-C analogues with respect to their anti-L1210 schedule dependency, Anhydro-Ara-C (O-2,2'-Cyclocytidine, monoacetate, NSC-129220) was subjected to intensive study because of its high degree of solubility in water. The observation that qd. Anhydro-Ara-C treatment is superior to qd. Ara-C treatment and approximately equal to q3hr., q4d. Ara-C treatment suggests that the duration of effectiveness per injection is greater for Anhydro-Ara-C than for Ara-C. That daily treatment produced long-term survivors among mice with early L1210 indicated that Anhydro-Ara-C given once each day provides active inhibitor concentrations and duration of effect sufficient to achieve total tumor cell destruction at sub-lethal doses. Assuming that the schedule dependency of Ara-C is due, at least partly, to its relatively rapid inactivation in vivo, the results are consistant with Hoshi et al's (Gann 62: 145, 1971) findings that Anhydro-Ara-C was not deaminated by mouse kidney pyrimidine nucleoside deaminase upon 3 hours incubation at 37°C whereas Ara-C was completely deaminated within one hour. Steuart and Burke (Nature New Biology 233: 109, 1971) have reported substantial increases in human leukemic cell deaminase levels associated with the development of resistance to Ara-C. It is possible that (a) Anhydro-Ara-C per se acts in a manner similar to Ara-C, (b) Anhydro-Ara-C is transformed to Ara-C in vivo, or (c) both. If the activity of Anhydro-Ara-C is due to transformation to Ara-C, the process is sufficiently slow to permit maintenance of maximally effective Ara-C levels when Anhydro-Ara-C is given once each day; thus, Anhydro-Ara-C would represent a soluble "reservoir" for Ara-C. If Anhydro-Ara-C exerts antitumor action without transformation to Ara-C, our finding that it is inactive against the Ara-C resistant subline, L1210/Ara-C, suggests that an increased deaminase level was not the major mechanism of resistance in this tumor variant, and that the mechanisms of action of Ara-C and Anhydro-Ara-C are similar. In such a case, it is possible that Anhydro-Ara-C is activated by deoxycytidine (CdR) kinase and that deletion of this enzyme is responsible for the cross-resistance of L1210/Ara-C. An Anhydro-Ara-C resistant subline of L1210 has been developed (by J. A. R. Mead) and we are planning to measure pyrimidine nucleoside deaminase and CdR kinase in both L1210/Ara-C and L1210/Anhydro-Ara-C.

3. B16 Melanoma and Lewis Lung (LL) Carcinoma.

Preliminary studies of innoculum level and tumor site influence on growth were performed to aid in standardizing these systems for use as secondary screens. Inasmuch as LL has shown variable results in hybrid mice, its growth characteristics in C57Bl/6 mice (the host strain of origin) are under investigation.

4. L1210 and P388 Leukemias.

In order to provide rapid feedback of information to the Drug Evaluation Committee and other program units, 33 agents were further evaluated against L1210 and 31 against P388. Excluding drug tests associated with B16 and LL developmental studies, 2,265 computer processible in vivo tests were conducted. Several tests involved materials new to the program that were of special interest because of their structures. Often these were

received in small amounts and were tested in-house to obtain the maximum useful data quickly.

Daunomycin (NSC-82151) was compared with 4 structural analogues against L1210. None of the related agents were as effective as Daunomycin. A new sample of Chromycin A_3 (NSC-58514) with twice the UV absorption activity as the old sample was found to elicit identical anti-L1210 activity. Hydroxyguanidine sulfate (NSC-44310), a proposed antitumor and antiviral material, was studied extensively to confirm reported antitumor activity. NSC-44310 was active against L1210 and P388, but the degree of anti-L1210 activity and the optimal dosage level is still under investigation. 5-Azacytidine (NSC-102816) had been reported to have a short half-life in solution. Studies of NSC-102816 comparing aged solutions with freshly prepared solutions showed no gross alteration in activity when solutions were kept for 12-48 hours. Carbazilquinone (NSC-134679) was compared with structurally related compounds for L1210 activity. None of the analogues was superior to NSC-134679. DL-Alanosine (NSC-143647) was found to meet DN-2A criteria against L1210. NSC-143647 was as active as L-Alanosine (NSC-526469). Ellipticine (NSC-71795) was compared with 9-methoxy ellipticine solubilized in hydrochloride and citrate buffer against L1210. Activity was greater using the citrate buffer and the 9-methoxy derivative was slightly more effective than ellipticine. We are proceeding with full schedule dependency studies of the citrate formulations. An extensive structure-L1210 activity study of DIC (NSC-45388) and selected analogues was conducted in collaboration with R. Engle. A number of Rifampicin analogues were tested both for antitumor activity and toxicity in vivo in preparation for anti-viral testing (with A. Goldberg). None showed antitumor activity against L1210 or P388. Anti-viral activity and activity against virus-induced neoplasms for derivations of Rifampicin are described in current Project Report, Serial No. NCI-3577.

5. Studies of the growth characteristics of mast cell tumor P815 have been initiated. P815 has been reported to exhibit an unusually long doubling time when implanted SC.

6. Some contract laboratories subject saline or water insoluble materials to sonification prior to screening in order to attain a suspension of minimal particle size. Although efforts are made to avoid heat generation during this process, heat could conceivably alter the structure of some drugs. Sixty drugs, both active and inactive, water soluble and insoluble, were either exposed to 30 seconds sonification or not sonified. These preparations were compared for activity against L1210 or P388. Sonification did not alter the activity of 85% of the drugs, increased activity for 10%, and decreased activity for 5%. These results were presented to the American Pharmaceutical Association in April, 1971.

7. During the year, Methanesulfon-m-aniside, 4'-(9-acridinylamino)-HCL (NSC-141549) passed DN-2A on the basis of its L1210 activity. The drug, however, is quite insoluble in both organic and aqueous solvents. The supplier (Dr. Bruce Cain) synthesized an analogue which, because of its solubility in organic solvents may be a more useful agent in that it may

enter lipid-like structures more readily, and cross the blood-brain barrier. The analogue, NSC-146434, is currently under investigation. An assay, involving extracting the material into ether, evaporating the ether and using the diazo coupling method of Schotten-Baum for color development and subsequent UV spectrophotometric readings, has been developed. The metabolism and distribution of this material will be investigated.

Proposed Course

Continuation of studies related to the development of improved assays for drug evaluation, structure-activity studies, studies of combination chemotherapy, and studies devoted to the solution of special problems arising during formulation development, toxicological examination, and clinical trial of active antitumor drugs is anticipated.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

Serial No. NCI-3583
1. Chemotherapy, DRD
2. Drug Evaluation Branch
3. Office of the Chief
4. Bldg 37

PHS-NIH
Individual Project Report
July 1, 1971* through June 30, 1972

Project Title: Studies on Modification of Division Patterns and Immunogenecity of Neoplastic Cells by Interaction of their Surfaces with Microenvironmental Macromolecules

Previous Serial Number: None

Principal Investigator: Muriel Lippman

Co-Principal Investigator: None

Cooperating Units: Microbiological Associates, Inc. (I. Kline), Experimental Therapeutics (V. Bono)

Man Years (NIH):

Total: 1 1/2
Professional: 1
Other: 1/2 (part-time)

* The principal investigator was appointed as of August 23, 1971.

Project Description:

Objectives:

1. The project aims at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells. To this end connective tissue acid mucopolysaccharides, which have been shown to block cell division in some cell types, as well as both natural and synthetic related polymers are to be obtained and tested for surface binding and cell division inhibition activity on a variety of cell types in culture.

2. Binding of a particular polymer to a cell surface depends on cell type but also on physiological age of the cell with respect to the cell and culture cycle. Conventional as well as new methods of cell separation are developed in order to investigate absorption as a function of the cyclic behavior of the cells.

3. In the case of manifest cancer the immune responses evoked by the malignant cells have failed to effect a complete rejection of the neoplastic cells. Evidence exists that attributes this failure to the weak nature of the cancer cell as an immunogen and to the phenomenon of enhancement frequently observed with sarcomas and carcinomas but not with leukemias. Studies are carried out with the aim of increasing the immunogenicity of the neoplastic cells and preventing some aspects of enchancement.

4. New methods are to be developed for rapid, microscale, in vitro tests suitable for screening small quantites of compounds and which provide more information than the degree of cytotoxicity alone.

Methods Employed:

Along with standard methods a substantial part of the project efforts are devoted to the further development of unique methods of cell separation, analysis, and culture which are needed to realize the aims and objectives of the project.

Major Findings:

1. Acid mucopolysaccharides of the heparin type have been shown to absorb to cell surfaces of a variety of cell types. In some cell types this absorption is accompanied by blockage of cell division. The increased negative charge of many neoplastic cells makes absorption of these highly anionic polymers difficult. In an effort to modify the cell surface so that it would be more susceptible to the polyanions, the effect of several cationic molecules was investigated. Two of these molecules yielded interesting results in terms of effects on the cells.

a. Spermine (NSC-69852) was selected because of its ability to bind heparin. In our studies spermine was shown to be cytotoxic to L1210 cells at concentrations of 0.2 $\mu g/10^5$ cells and above. Cell division was completely prevented in a 24 hour period at a concentration of 0.1 $\mu g/10^5$ cells. Cell viability was 100% at this and lower concentrations of spermine as indicated by Trypan Blue exclusion. Concentration of 0.05 $\mu g/10^5$ cells was ineffective in preventing cell division. All concentrations produced an increase in cell volume. Cytochemical staining with Feulgen reaction for DNA showed unusual condensation and distribution of DNA in the cells at 0.1 $\mu g/10^5$ cells. At this concentration staining with Methyl Green-Pyronin for DNA and RNA respectively, suggested an increase in content of RNA. ^{3}H-thymidine and ^{14}C-valine incorporation studies (with V. Bono, Experimental Therapeutics) measuring DNA and protein synthesis are summarized below. These data show that DNA synthesis was inhibited significantly at the division blocking concentration and that protein synthesis was also inhibited but later than DNA synthesis. At the higher concentration of spermine the same lag in inhibition of protein synthesis was observed at a time when cells still appeared viable. The effective concentrations obtained in these experiments are at least ten fold lower than the lowest reported by other investigators.

^{3}H-Thymidine and ^{14}C-Valine Incorporation into DNA and Total Protein in L1210 Cells Treated with Spermine

	Time Interval (Hours)			
	0-1.5	1.5-3	3-24	
Concentrated Spermine $\mu g/10^5$ cells	(Incorporation expressed as % of controls)			
0.05	113	77	99	DNA
	107	98	102	Protein
0.1	97	65	60	DNA
	103	91	75	Protein
0.2	90	36	1	DNA
	100	71	0	Protein

Tests in the primary screening program in 1962 and 1963 showed spermine to be inactive in non-toxic concentrations against Sarcoma 180 (inj. days 1-7) L1210 (inj. days 1-10), and Walker 256 (inj. days 1-5). In vitro testing against 9KB gave ED_{50}'s of 1.2-4.8 $\mu g/ml$.

In the light of studies reported after 1963 on the mechanisms of action of spermine in which the spermine is believed to be modified by spermine oxidase found in some sera (fetal calf and calf) and not in others (e.g. horse) it seems advisable to test the effect of spermine on cells grown in serum free medium as well as L1210 cells grown in horse serum and in the presence of ascites fluid.

The need for activation of spermine by serum factors or conversely its rapid inactivation might suggest more appropriate schedule dependency trials in vivo.

b. The second cationic substance tested was polylysine. This polymer is known to bind to cell surfaces. Polylysine. HBr was obtained from Miles Laboratories in two molecular weights, one with an average molecular weight of 68,000 and the other with an average molecular weight of 14,200. The higher molecular weight polymer prevented cell division completely at doses between 20-30 $\mu g/10^5$ cells while the lower molecular weight required 40-50 $\mu g/10^5$ cells to produce the same effect in a 24 hour period. No evidence of aggregation was found at the effective blocking concentration.

Polylysine (NSC-49841, m. w. unspecified) was tested in the primary screening program in 1960 against Ca755, L1210, and Sarcoma 180. The highest dose tested, 25 mg/kg, was non-toxic and inactive.

Since polylysine coats surfaces of cells it is possible that the observed in vitro blocking of cell division may be effectively combined with an immune response directed against polylysine coated cells. Such experiments are being carried out

2. The ability of selected polymers to absorb to the surfaces of neoplastic cells and hence modify their immunogenicity can be tested by measuring the change in rate of tumor growth or rejection following transplant of cells treated with the polymer into hosts of varying histoincompatibilities. In order to initiate these studies three ascites tumor lines, all carrying the H-2^a specificities were obtained from Dr. T. Hauschka (Roswell Park Memorial Institute) and carried here in A/He mice. The Lymphoma #2 TA3Ha (HA=Hauschka) and TA3Kl (Kl/Klein) were selected because of their narrow spread of survival times at particular inocula, and because the TA3Ha line which now transgresses histocompatability barriers, can be used in comparison with the TA3Kl line which retains its H-2^a specificity. Median survival times of all three tumors in A/He mice are shown on the table below. In addition the median survival times are shown for the TA3Kl tumor in animals in which immunosuppression was obtained by radiation 24 hours prior to tumor inoculation. The upper radiation dose tolerated by these animals was 400 rads. The immune depressed animals showed shorter survival times and more rapid tumor growth than the non-radiated control animals at the same cell inoculum. These results are indicative of an immune response to the tumor and enable us to use this system to measure modification of immunogenicity with respect to H-2 antigens in allogenic transplants and with respect to tumor antigens in syngeneic transplants. These studies are carried out with Dr. I. Kline, Microbiological Associates, Inc.

Median Survial Time (Days) for Lymphoma #2, TA3Ha and TA3Kl Tumors at Various Inocula

	Tumor Cell Inoculum					
Tumor	10^6	10^5	10^4	10^3	10^2	10^1
Lymphoma #2		13	15	15	18	
TA3Ha			10	15	15	17
TA3Kl	11	12	16	16		
TA3Kl 350 rads	7	8	10	11	14	17
TA3Kl 400 rads	7	8	8	10	11	17

3. Studies were begun with various sublines of B16 melanoma. B16 cells cultured in Eagle's medium, medium 199, RPMI 1630, and Ham's F10 showed amelanotic and melanotic cells in the same culture, but in different proportions in the different media. In addition, some media seem to favor flattening of the cells and some clones in the population appear to be contact inhibited. These contact inhibited cells have not yet been isolated in sufficient quantity to inject back into animals to determine whether they retain their neoplastic character. Our observations show that variations in form, ability to make melanin and to attached to the substrate can all be due to variations in the medium used. Reports that such changes result from treatment with BUDR and cyclic AMP may represent selection pressure for changes which occur spontaneously in the culture rather than uniform changes induced in the population by the

agents.

Significance for the Program:

1. Focus on the cell surface as a target for chemotherapeutic agents extends the search for active materials to include compounds which

a. have good possibilities for achieving selectivity in cell division blocking between normal and neoplastic cells.

b. can be used to increase the immunogenicity of neoplastic cells and therefore, may be used in combined chemo-immunotherapy. Such an approach insures a more complete attack on the malignant population than is afforded by chemotherapy alone.

c. are involved in the normal regulation of cell division in vivo. This approach recognizes that manifest malignancy is a function not only of a transformed cell population but of a permissive microenvironment of the cell.

2. Development of special methods for obtaining unique subpopulations of a given cell type would provide a good tool in the search for agents which would be expected to effect cells in prolonged G_1 or other chemotherapeutically recalcitrant subpopulations.

3. Good in vitro methods on a micro scale which permit testing of agents in small quantity on a variety of malignant cell lines would provide a useful adjunct to the general KB cell test and enable a search for molecules which may affect one specific cell type and not others. The same endeavor in in vivo systems would be prohibitive in terms of the number of animals required, length of time required to get the data and quantity of test agent available The problem of finding in vitro assays which correlate well with in vivo activity is especially acute where only modest amounts of drug are available and a variety of neoplastic cell types should be studied. Such in vitro systems enable one to sort out direct effects on cell division from immunological and pharmacological problems involved in obtaining effective agents. While agents that are effective when tried in vivo directly are good candidates for further analysis and study, it is also very likely that compounds which do not work in vivo fail to do so for multiple reasons which could be overcome if attacked individually. Such agents are now eliminated from further study and could include many potentially active materials.

Proposed Course:

The project at this time is in its initial stages since the appointment of the principal investigator began only in September 1971 and the first several months were spent in setting up the laboratory. The proposed course will be developed along the lines described under project description and objectives.

ANNUAL REPORT

PROGRAM ANALYSIS BRANCH

DRUG RESEARCH AND DEVELOPMENT, CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

PRA provides counsel and assistance to all parts of DR&D in the capture, maintenance, analysis, and dissemination of technical information and data. The Branch maintains the DR&D chemical and biological data processing systems and provides information for program-monitoring and decision-making. Other activities include systems management and the publication of program and program-related information.

These functions of the Branch are carried out by four sections whose work is augmented by several contracts. The Data Processing and Computer Systems Development Section is primarily concerned with the design and development of an extended chemotherapy chemical information system. The Section's responsibility for the operation and management of the biological data processing system was transferred to the Office of the Chief, PRA, to allow the Section to concentrate entirely on this work.

An information storage and retrieval system is operated by the staff of the Literature Research Section; this section collects the published literature on cancer chemotherapy and prepares data summaries, special reports, literature searches, and annotated bibliographies specific to the needs of cancer chemotherapy investigators. In addition, the Section maintains on contract the current awareness-serial Cancer Chemotherapy Abstracts (CCA) and operates the Chemotherapy Library.

The Publications Section produces Cancer Chemotherapy Reports (CCR), a primary-source journal containing clinical and experimental research on chemical agents developed in the Chemotherapy Program by contractors, collaborating institutions, and other investigators. The Section also publishes supplements to CCR on screening data and program information, assists the chemotherapy staff with special publications, publishes the Chemotherapy Fact Sheets on drugs, maintains the mailing lists for its own publications and CCA, and clears contractors' manuscripts for publication.

The Scientific Records Section serves as the focal point for the maintenance of chemotherapy scientific records, is responsible for the storage and retrieval of chemical and related information on all synthetic compounds and plant extracts received for screening, maintains a coordinate index information retrieval system for substructure search, and performs special searches as requested by the staff.

SUMMARY OF PROGRAM ANALYSIS BRANCH ACTIVITIES

PRA is continuing work on the functional design of an extended chemical information system intended to handle chemical structure information and to manage the inventory of compounds. A new contract was awarded to A. D. Little, Inc., for improvement and expansion of the Drug Research and Development Biological Data Processing System. Herner Information Services, Inc., was awarded a contract to prepare, print, and distribute Cancer Chemotherapy Abstracts (Volumes 13 and 14 for 1972 and 1973). In FY72 the work of the Branch was supported by the following contracts: Chemical Abstracts Service (PHS-43-1021); Control Data Corporation (PH43-67-1356); George Washington University (NIH-69-2116); NYU Medical Center (PH43-67-1336); John I. Thompson Company (NIH-69-2158); Scientific Literature Corporation (NIH-71-2306); University of Pennsylvania (NIH-71-2187); and Vitro Laboratories (NIH-69-58).

The principal activities of the Branch relate to program analysis, communications, and systems management.

A. Program Analysis

1. The Quarterly Statistical Report, summarizing the flow of materials into the screen and their progress through various decision networks into clinical trial, was produced on schedule. Internally, Branch personnel reviewed these statistics against progress in previous quarters, bringing any significant changes in trends to the attention of the Associate Scientific Director for DR&D.

2. The report analyzing the costs of supplying commercial drugs to grantees and others was improved and revised. Monthly and quarterly reports of shipments were prepared on schedule. Although the need for tight control of ceilings was obviated, an increase of 40% in drug shipments and increased interest by Chemotherapy in cost accounting caused stepped-up demands for special queries. These involved: (a) all shipments of a specific compound, (b) shipments to a specific investigator, or (c) previous monthly costs to be used in forecasting future demand. Approximately $1,000,000 worth of commercial drugs were shipped by the Program, not including bulk drug costs.

3. Many queries of the Biological Data File were made during the year to assist DR&D staff. For the Drug Development Branch (DDB), the activity of various nucleosides was determined as well as the number of compounds (and their activity) supplied by grantees of the Medicinal Chemistry Study Section. Lists of plants collected in various countries were also provided upon request. For the Screening Section, Drug Evaluation Branch (DEB), queries were made in order to schedule testing, to check on results and to make sure that testing had been performed in development of the new screening protocols, to aid in the analysis of slow-growing solid tumor systems such as B16 melanoma and Lewis lung carcinoma, and to provide optimal dosage information on analogs for the Drug Evaluation Committee. Since the results of the queries provide specific information, much staff time is saved by obviating the need for review of the full Screening Data Summary.

4. Recognizing the difficulties to be expected from the expansion of the screening program, PRA worked with DDB and DEB to facilitate the acquisition and flow of materials. As a step toward a system that would allow monitoring compound input by source, PRA instituted several studies during the year. The synthetic file was analyzed to determine the total number submitted by a supplier, the geographic distribution of suppliers to aid in collection of compounds, the type of organization, and the volume of compounds submitted each year.

5. The acquisition of 3,486 unique samples from Walter Reed by computerizing methods from December 1969 through March 1972, has encouraged several projects, jointly undertaken by several PRA sections or in cooperation with DDB, to evaluate the use of magnetic tape data bases for compound acquisition. The impressive results of experimental substructure searches against the 2,000,000 compound CAS file and the 750,000 newly synthesized compound file of the Institute of Scientific Information (ISI) (each increasing at a yearly rate of several hundred thousand) has resulted in planning a full field test for using this approach on the ISI file for selective acquisition. Our experience with Kodak indicates that potential suppliers who are not prone to furnish random samples may be prepared to submit analogs of compounds of interest. Another experimental project is the evaluation of mechanized means to identify active experimental or clinical compounds reported in Cancer Chemotherapy Abstracts which are not in the Chemotherapy collection.

B. Systems Management

1. The library of Screening Data Summaries from the biological data processing system was converted to microfilm via computer (COM). This process markedly reduced overcrowding in the Screening Section, DEB; has resulted in easier retrieval of information by staff; and has decreased the amount of filing time required of the Scientific Records Section. This is an example of how keeping up with advances in technology and DCRT services enables us to take advantage of modern methods.

2. Monitoring the computer expenses incurred at DCRT is a continual responsibility of the Branch, and four special analyses were prepared during the year with options presented for reducing costs. To increase our control over expenses before they are formally reported to us. Dr. Greenfield developed a method for analyzing the DCRT accounting tapes by programmer or account number to provide detailed listings of jobs submitted, route of submission, and so forth.

3. PRA has initiated a major contracting effort which will analyze present uses of, and the structure of, the biological data processing system. This should allow us to modernize and streamline the system, to make it more flexible, and to provide more useful information.

4. The Branch has continued its efforts to give scientists in the program maximum control over their data and is establishing small files con-

taining subsets of data from the biological data processing system to permit easier access to information from which structure-activity relationships may be deduced.

5. The Data Processing and Computer Systems Development Section concentrated on the development of an extended Chemotherapy chemical information system. The section monitored the efforts of the University of Pennsylvania, under contract, to analyze the Program's chemical information needs and prepare detailed design specifications for a system to be implemented over a two-year effort starting in early FY73. This system will provide for improved techniques to: (a) determine whether newly received compounds are new to the Program, including the identification of compounds and moieties of interest; (b) automatically assign samples to screeners and output communications to suppliers, screeners, and Chemotherapy staff, including shipping lists and chemistry cards with structural diagrams; (c) maintain on-line inventory control and monitor the replenishment procedures; (d) develop more comprehensive data files with simplified procedures to search cross-files and subfiles and produce on-demand, custom-tailored reports; (e) characterize compounds by substructure search with simplified inquiry procedures, structure output, and linkage to the other data files; and (f) automatically maintain operational and management control over the system. During the analysis phase of the design effort, areas of the current system that could be streamlined were identified, including the elimination of packing and supplier lists and most finished chemistry and flexowriter cards. Changes in these procedures are now being tested. In addition, the programming was completed to bring the Chemical Abstracts Service's substructure search system in-house by outputing an NSC number rather than an NCI registry number.

6. The function of assigning systematic names was transferred from DDB to PRA on a trial basis in an attempt to group functions more logically and to speed paper flow.

7. In addition to its routine duties, the Scientific Records Section made 117 substructure searches at CAS with 19 updates of old searches. These included three large-scale structure-activity studies of the 1,4-quinones, the nucleosides, and the folic acid antagonists. They also cooperated with Dr. John Driscoll of DDB in creating a machine-manipulatable file of summarized data for the 1,4-quinones.

C. Communications

1. An informal publication effort was initiated by sending a newsletter to all DR&D suppliers and screeners. The response to this was most gratifying, indicating a strong need by many of the suppliers to feel a greater sense of participation in the Program. Because of the information, many former suppliers have expressed plans to become active again in sending in samples.

2. In its 14th year of continuous publication, Cancer Chemotherapy Reports (CCR) was considerably improved in format and appearance. The Publications Section produced five issues of CCR Part 1 (621 pp), one issue of CCR Part 2 (246 pp), and one issue of CCR Part 3 (104 pp) in CY71. The Section also prepared the Proceedings of the Chemotherapy Conference on Procarbazine (Matulane), assisted in the preparation of revised screening protocols and two booklets showing chemical structures of interest to the program, and updated and revised the Proceedings of the First Joint Working Conference of the NCI Chemotherapy Program (CCR Part 3, Oct. 1971), for inclusion in the Progress Report of the Chemotherapy Program, NCI. Papers are in preparation for a third volume of CCR Part 3. A total of 161 contractor manuscripts were cleared for publication in 1971.

3. Cancer Chemotherapy Abstracts was brought essentially up-to-date and is to resume monthly publication in June 1972.

4. Through a cooperative agreement with the National Library of Medicine to utilize the ELHILL (MEDLINE) data base, the Branch has been provided with direct access to a major segment of the MEDLARS input. A recently installed terminal, providing on-line access to articles in over 1,000 recently published journals, is expected to assist members of the Literature Research Section in updating the Section's clinical files and in answering requests. Monthly citations from DCRT's CBAC tapes and recurring MEDLARS bibliographies from the National Library of Medicine facilitate the search for new agents.

BRANCH STAFF

PRA is composed of a staff of 34 persons consisting of three professional senior staff members (GS-13 and above), nine other professional staff members (GS-9 and above), two PHS Commissioned Corps Officers, 19 technical clerical staff members, and one Stay-In-School person.

Chief, Program Analysis Branch	Barbara R. Murray
Assistant Chief	Rhoda R. Mancher
Special Assistant	Alan J. Greenfield, M.D.
Head, Data Processing and Computer Systems Development Section	Sidney Richman
Acting Head, Scientific Records Section	George F. Hazard, Jr.
Acting Head, Literature Research Section	Dorothy Algire
Acting Head, Publications Section	Pamela Hutchins

HONORS AND AWARDS

1. Barbara R. Murray served as Chairman of the COSATI Task Group on Chemical Information Systems.

2. Mrs. Murray served on the Steering Committee for the International Cancer Research Data Bank.

3. Mr. George F. Hazard, Jr., was named to serve on the Editorial Advisory Board for Current Abstracts in Chemistry/Index Chemicus.

4. Miss Barbara Quarles, Stay-in-School student, Scientific Records Section, received a cash award for outstanding performance during 1970 and 1971.

PUBLICATIONS AND PRESENTATIONS

1. Goldman, G., Fortner, C. L., Cradock, J., Murray, B., Hsieh, R., Thompson, W. L., and Block, J. B. A computer based medication system for cancer chemotherapy patients. American Journal of Hospital Pharmacy, April 1972.

2. Hazard, G. F., Jr. The use of the CAS substructure search system on a large drug development chemical file. Presented at the 162nd National ACS Meeting, Washington, DC, September 13, 1971.

3. Murray, B. R., and Hazard, G. F., Jr. Use of the Chemical Abstracts registry system in a drug development program. Presented at the National ACS 7th M.A.R.M. Meeting, Philadelphia, Pennsylvania, February 14-17, 1972.

4. Shamos, M. I. Guidelines on file backup. Interface 27:3-6, 1971.

5. Shamos, M. I. Blocking factor reflects cost. Interface 30:9-10, 1971.

6. Raciti, A., Greenfield, A. J., Skinner, D. B., Newman, M. H., and Rutherford, R. B. Organ blood flow by radionuclide-labeled microspheres during mechanical ventricular assistance. Annals Thoracic Surgery 11:43-50, 1971.

7. Murray, B. R., and Olejar, P. The nature of system requirements and desirable characteristics for handling drug data. Presented at the Computer-Based Information Systems in the Practice of Pharmacy Meeting, Chapel Hill, North Carolina, July 19, 1971.

8. Greenfield, A. J. Demonstration of a drug information system, with NCI Nursing Fact Sheet information. Presented at the Computer-Based Information Systems in the Practice of Pharmacy Meeting, Chapel Hill, North Carolina, July 19, 1971.

9. Murray, B. R., Wooster, H., Wilson, K., Shull, H., Harris, G. H., and Olejar, P. Problems of reviewing R&D grant and contract proposals. Panel presentation at the 163rd National ACS Meeting, Boston, Massachusetts, April 11, 1972.

10. Mancher, R. R. Data processing changes. Presented at the Screening Contractors Meeting, Hazleton Laboratories, Vienna, Virginia, April 14, 1972.

11. Murray, B. R. Letter to the Editor: Cancer Journals. Chemical and Engineering News, January 31, 1972.

I. Systems Management

The Drug Cost Reporting System was stabilized during this fiscal year, and many procedures were changed to increase the efficiency of data capture by the system. New report formats were added and older reports were modified to increase their flexibility. Some relaxation in budgetary restrictions obviated the need for tight controls of amounts spent by cooperative groups for drugs; on the other hand, increasing interest by the Chemotherapy Program in cost accounting caused stepped-up demands to be made on the system for special queries. For the most part, response time for special queries was reduced to 24-48 hours, and in some cases requests for special information received in the morning were responded to by the close of business on the same day. Due to staffing difficulties, we were unable to transfer the former investigator address file to DCRT operation, but maintenance of records for this file has continued so that the transfer can be made if personnel become available.

Much effort was expended this year in improving the data content of the Drug Shipment Files. This has greatly increased the accuracy of reporting to the Program.

Reporting periods were standardized to include:

- A monthly report of drugs shipped to cooperative groups broken down by NSC number and a similar report broken down by investigator within the cooperative group.
- A monthly report of all drugs shipped from DR&D.
- Quarterly reports including the two reports mentioned above.
- A breakdown of drugs supplied to intramural investigators.
- A breakdown of drugs supplied to contract investigators and a detailed breakdown for each investigator every 6 months of all drugs he received, including the quantity and cost of each individual lot number shipped to him. Accompanying this report for the use of the group chairman is a slightly less detailed report by an investigator showing the NSC number and cost of each drug shipped to each investigator.

Special queries were run for several cooperative groups who were concerned about the shipments to a specific investigator. Special queries were also made for the administrative staff to determine on a month-by-month basis the cost of all drugs shipped from the Program to aid them in forecasting requirements for the remainder of FY72 and for FY73.

DR&D Biological Data Processing

The major responsibility for the DR&D biological data processing system was transferred to the Office of the Chief during this period. The integrity of the file was enhanced and some aspects of the routine processing were modified in the interests of efficiency, economy, and utility.

In the interests of rapid reporting, the computer production run was returned to a weekly schedule at the beginning of FY72, as the capacity of the screening laboratories was increased.

Programs to produce statistical reports on a weekly, monthly, quarterly, and year-to-date basis were developed.

Graphic presentations of schedule-dependency data were produced automatically on a routine basis.

Combination chemotherapy testing results were put on a regular bi-weekly production schedule. Reports were mailed routinely to screeners and staff. A cross-reference index was prepared as automatic output of the program.

Automated evaluation and editing of the screening data was inaugurated into the biological data processing system in compliance with the new screening protocols established by the Drug Evaluation Branch (DEB). Some of the burden of reporting was removed from the screening laboratories as a result of this improvement to the system.

Fifty-six queries to the master files and the plant header files were performed during this period. These queries made information on the file available to requesting segments of the Program on a timely basis in an easy-to-read format. Some of the projects they supported were:

- Plant collection in various parts of the world.
- Scheduling of screening (primary and secondary).
- Compounds supplied by a particular person or institute or extracted by a particular laboratory.
- Materials supplied by grantees.
- Activity of nucleosides--in preparation for a meeting on structure-activity relationships.

- Tumor systems tested in support of the screening protocol.

All cell culture testing during the period of more stringent criteria is being re-evaluated for activity.

II. New Projects

A. Combination Chemotherapy

A summary file of combination chemotherapy data is being established for rapid query. This file contains optimal dose and highest non-toxic dose information as well as a description of the test system. The method of access is such that no knowledge of computer programming is necessary to query the file.

B. Schedule Dependency

A similar system has been established for schedule-dependency data. This system provides information on the optimal dose citing route, regimen, increase in lifespan, and survivors at that dose and at the highest nontoxic dose. At this writing the system allows retrieval by NSC number only, but other options will be added as the system progresses. A similar type of summarization of primary screening with interactive capabilities will extend the usefulness of the DR&D files for immediate inquiry and allow users to manipulate their data more rapidly and completely than is presently possible with the tape-oriented systems now in use.

C. Biochemical Testing

An analysis of the requirements and best methods for including biochemical testing on the primary screening files was undertaken during this period in the Office of the Chief. At present, the data are being prepared for entry on the file and will shortly be appearing on the screening data summaries. These data will henceforth also be available for querying either alone or in conjunction with other *in vivo* and *in vitro* testing.

D. AKR Lymphoma Data

In conjunction with Southern Research Institute, analysis is underway to capture and report on the AKR lymphoma data. We expect this file to be processed separately, as in the case of combination chemotherapy data, with reference made on the master files. At the same time, the Moloney virus data are being investigated for the same purposes.

E. Updating of Communications with Screeners and Suppliers

PRA increased dissemination of information to suppliers and screeners of compounds for the DR&D screen. Many responses con-

tained valuable suggestions for improvements in our information formats and for new types of information which might be useful to those outside the Chemotherapy Program. At present, the staff of the Office of the Chief is evaluating the responses in order to formulate a set of positive recommendations that may be used by the Program.

F. Plant Products

A file of plant products collected more than 10 times, and those that have been confirmed in the screen, was established for the purpose of rapid positive verification of duplicates from the USDA.

G. Microfilm of the Screening Data Summary Library

The library of Screening Data Summaries from the biological data processing system was converted to microfilm via computer (COM). This is a new method for microfilming and while it was somewhat experimental, it resulted in clear microfilm reproduction without printing thousands of sheets of paper beforehand. We plan to use this method for all our large files in the future, especially since the equipment will soon be available at the Division of Computer Research and Technology.

The introduction of microfilm into the Screening Data Summary library has reduced overcrowding, decreased filing time, and facilitated retrieval.

III. Clinical Data Processing

At the beginning of the fiscal year all the responsibility for clinical data processing was transferred from PRA. All programs and documentation were turned over as well as all data files. Due to minimal demand and difficulties in updating the files, the Nursing Fact Sheet was dropped, although all programs and data were saved on magnetic tape for possible future use. The Clinical Pharmacy has, however, continued to provide some service in this area, and any material we had received prior to making the decision to eliminate Nursing Fact Sheets was transferred to them.

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

Program Analysis Branch

To permit concentration on the design and development of an extended Chemotherapy chemical information system during FY 1972, this Section's responsibility for the operation and management of the biological data processing system was transferred to the Office of the Chief, PRA. Similarly, the responsibility for actively monitoring the contract with Chemical Abstracts Service (CAS) for the registration and substructure searching of the Chemotherapy compound collection was transferred to the Scientific Records Section. The Section, however, continued to provide consultation in these transferred activities.

In directing the effort to develop an extended chemical information system, the Section supports the Chemotherapy Decision Network by providing improved methodology in the following areas:(1) Stage I, steps 2, 3, and 4--identifying duplicates, analogs of compounds of interest, and drug abuse and hallucinogenic compounds, and the storage, retrieval, and dissemination of chemical information for these compounds; (2) Stage I, step 5--assigning NSC numbers on a computerized basis and maintaining the inventory control; (3) Stage IIA, step 1--automatically assigning special tests for analogs of compounds of interest and providing information necessary to designate the optimum use of duplicate samples; (4) Stage IIA, step 3--automatically distributing agents to testing laboratories based on backlogs. The new system will provide improved operational and management control of all steps in Stage I. In addition, the Section supported Stage I, steps 1 and 2, by testing the value of using machine-processable tape services and computerized data bases of commercial compounds to identify for acquisition action newly synthesized analogs and on-the-shelf compounds that are not in the Chemotherapy collection.

A contract was awarded to the University of Pennsylvania to design and prepare the detailed specifications necessary to implement the extended Chemotherapy chemical information system. Building on the preliminary study made by CAS in April-June 1970, the University of Pennsylvania conducted a detailed system analysis and submitted an overall (functional) system design with viable options, each having varying capabilities to satisfy the required and desired objectives of the system. Technical advice and consultation in evaluating the alternate designs proposed were provided by an ad hoc committee to the DR&D staff. This committee, consisting of key DR&D staff members and representatives from Federal Agencies, industry, and universities who have had extensive experience in large data processing systems and particularly in chemical information systems, recommended the award of the contract to the University of Pennsylvania and agreed to provide assistance throughout the design and implementation phase.

Recognizing the constraints of time and money, the contractor is using an engineering approach in the design of the system. The modular capabilities of many existing chemical information systems in Government and industry were examined to identify those which would be applicable to our needs and would

be transferable, without change or after an acceptable level of effort, into the new system's environment. Having received acceptance of the overall system design, the University of Pennsylvania is proceeding with the detailed functional design. When this is accepted, the detailed specifications and the planning document for implementation and testing will be delivered in early FY73.

Implementing and installing the design system at DCRT will be a two-year effort, estimated to cost about $800,000. Chemotherapy Program Staff has granted preliminary approval for funding the first year of the implementation effort at a level of $518,000. The new Phase I system, with somewhat fewer capabilities than the final system, will be tested in parallel with the existing, semiautomated system during July-September 1973. The total Phase II system is expected to be operational in July 1974.

This new system will provide for the (1) rapid, economical registration of compounds, resulting in substantially increased through-put to screeners and rapid response to suppliers; (2) ability to absorb sudden increases in samples with current manpower without backlog; (3) rapid, user-oriented, and economical substructure search for single and multiple inquiries with chemical structure output; (4) on-line access to inventory status with control of inventory transactions; (5) operational control of the system flow, including automatic notification of problem cases; (6) capability to interact structural, textual, and inventory data with links to the biological file; (7) management control of the system with recurring and on-demand reports; (8) elimination of overlapping manual and automated procedures and the automatic preparation of documents such as chemistry cards, shipping lists, bottle labels, supplier acknowledgment, etc.; and (9) cost-effective handling of chemical information, and the rapid expansion to an interactive, time-sharing system.

During the analysis phase of the design effort, recommendations were made to streamline the current, semiautomated procedures. We are now testing the elimination of packing lists, supplier lists, and most finished chemistry and flexowriter cards. This will permit the reduced staff to process new acquisitions and to support the biological data processing system in several critical areas in anticipation of a possible change in the contractor operating that system.

To benefit from the experience of designers and managers of other large chemical information systems, three seminars were conducted. One dealt with structure input and display methods; a second dealt with Imperial Chemical Industries' CROSSBOW system; and the latest was designed to evaluate the screens proposed for the improved substructure search system.

The University of Pennsylvania is providing programming support in cataloging and linking the final module of the CAS substructure search system to permit operation at DCRT. This will insure increased responsiveness to requests for analog searches.

A computerized batch control system was developed to monitor the processing of new samples, pending the implementation of the extended chemical informa-

tion system. This system can provide information on the status of the samples from receipt through registration at CAS and the assignment of NSC numbers, as well as automatically prepare supplier and screener communications.

With the cooperation of the Drug Development Branch (DDB), various Sections within PRA are evaluating the potential use of chemical data bases and systems outside the Chemotherapy Program to facilitate compound acquisition. The Institute of Scientific Information (ISI) offers a unique opportunity for selective acquisition since their experimental service--Automatic New Structure Alert (ANSA)--can be used for substructure searches of new compounds reported in the journal literature from the fields of organic, biochemical, bioorganic, pharmaceutical, and agricultural chemistry. From 1968 to 1971, literature references for 750,000 new compounds have been stored and the files updated monthly with 14,000 new compounds. A query consisting of 22 search questions was made against a monthly tape for moieties of interest to the Program. The analogs requested were those of folic acid, platinum amine complexes, and guanazole. Of the 229 analogs reported, only 4.6% appeared in our collection. It is significant to note that while 34% of the analogs were reported by investigators in domestic organizations, all of whom are our suppliers, none of these compounds were made available to the Program. The Program's foreign liaison offices could be particularly helpful in acquiring compounds from foreign sources. A more extensive test is being prepared for analog searches of compounds: (1) reported in the DDB brochures entitled Some Chemical Structures of Current Interest to the Chemotherapy Program and Drugs Active in Clinical Cancer (distributed at the September 1971 ACS national meeting); (2) represented by other selected agents in Decision Network 3 and above; and (3) currently being synthesized under contract. Of course, the effectiveness of this selective-acquisitions approach will be determined only by visits and communications to new and current suppliers, both domestic and foreign.

Similar selective acquisition experiments are under way at Eastman Kodak and Shell Development Companies. Chemical information specialists at these organizations are currently preparing to conduct searches of their collections to identify analogs to the structures reported in the above-mentioned brochures. Organizations reluctant to furnish random compounds have indicated an interest in submitting analogs of compounds of interest to the Program because of the higher probability of activity.

A structure tape of approximately 5,800 Eastman Kodak open compounds was automatically matched for duplicates in the Chemotherapy file to identify potential compounds for acquisition. Of these compounds, 41.6% were not in our collection and have now been identified to facilitate acquisition.

A related project is the test of Imperial Chemical Industries (ICI) CROSSBOW System to convert a computerized chemical structure represented by a Wiswesser Line Notation (WLN) to a CAS connection table. If successful, this technique would permit the Program to machine-check for duplicates other automated files of cataloged compounds (such as Aldrich Chemical Co.) where structures are represented by WLN. Further, it may permit the Program to machine-check for acquisition purposes the holdings of potential industrial and Governmental suppliers, the structures for which are stored in a WLN file. When last

reported in 1968, these files (excluding ISI's) totaled 927,000 compounds, representing the holdings of pharmaceutical manufacturers such as Hoffman-LaRoche, Inc., Eli Lilly & Co.; and Winthrop Laboratories, and Governmental agencies such as Fort Detrick and Edgewood Arsenal's Industrial Liaison Office. Because of input problems, the preliminary efforts to convert the Aldrich Chemical Company's catalog in machine-processable form from WLN to the CAS connection table were not successful. However, ICI has been requested to demonstrate this reported capability by using a monthly ISI tape. The technique would be very valuable in any effort to exploit in depth the ISI tapes which report newly synthesized compounds.

Another project which is being completed is the evaluation of building a computerized, searchable data base of Cancer Chemotherapy Abstracts (CCA) by using the information captured as a by-product of producing camera-ready copy by a magnetic tape selectric typewriter. A test file for the 1968 volume has been built by reformating programs and it is searchable by the CBAC search system maintained by DCRT. Preliminary results on a small test file indicate a significantly higher recall rate by using computerized searches than by using the manual index. More extensive testing of the entire 1968 file is scheduled. Information for CCA volumes from 1967 to 1970 is available in a machine-processable form. In December 1972, volumes for 1971 and 1972 will be available for inclusion in machine-searchable data base. The Lister Hill National Center for Biomedical Communications and NLM's Toxicology Information Program have expressed an interest in including the CCA data base in their literature search systems.

Another joint, experimental project is the evaluation of using mechanized means to identify experimentally or clinically active compounds reported in CCA which are not in the Chemotherapy collection. Using the CAS NAME MATCH program, the names of compounds reported in one issue of CCA were matched against CAS's file to secure CAS Registry Numbers, structures, and synonyms. Some 66% of the names retrieved CAS registry numbers, the structures for which are now being matched by machine methods against the Chemotherapy collection. A higher ratio of CAS registry numbers would be retrieved if there had been a prior review by a nomenclature chemist of the names reported in CCA.

An evaluation project was defined to assess whether Cancer Chemotherapy Reports and CCA have met the communications needs of the cancer chemotherapy research community in disseminating clinical and preclinical chemotherapy research information developed within the Program and by other investigators. The objectives and methodology of the study were delineated.

The Section also coordinated all information required by NIH, DHEW, and GSA for the Automated Data Processing and Management Information Reporting System.

During FY73, the Section will continue to support the implementation of the extended chemical information system and the development and exploitation of data bases in the chemical and literature area.

LITERATURE RESEARCH SECTION

Program Analysis Branch

The Literature Research Section provides the Chemotherapy staff with information needed for Decision Network review, for meeting FDA requirements for IND filing, and for the preparation of clinical brochures. Information is obtained from the world's published literature on chemotherapy and from NCI Contract Progress Reports. Computer services provided by the National Library of Medicine and the Division of Computer Research and Technology assist with the searching. The Section's extensive reprint file, indexed by disease and type of agent, supplies readily available original data.

I. Literature Services

The Section filled approximately 135 requests for literature searches during the year. Information was generally submitted in the form of bibliographies and copies of articles. Annotations were provided as time permitted. Among the more extensive bibliographies prepared were those on the clinical applications of rifamycin SV, the clinical use of methotrexate (1946-1971), and the use of chemotherapeutic agents in the surgical adjuvant treatment of sarcomas, melanomas, tumors of the head and neck, and cancers of the breast, colon, lung, stomach, bladder, ovary, cervix, prostate, and endometrium. Other interesting or comprehensive bibliographies included those on bleomycin; cis-dichlorodiamine platinum and other platinum compounds; camptothecin; neocarzinostatin; the chemotherapy of pancreatic cancer, chondrosarcoma, and rhabdomyosarcoma; and combination treatment of Hodgkin's disease. Updated bibliographies were prepared on chromomycin A_3 and dibromodulcitol. A long-range project to search the Section's reprint file for all articles on the treatment of breast cancer continues.

Other on-going projects include the updating of clinical files, the testing of commercially available tape services, and the continuing search for new or potential antineoplastic agents. The Section's clinical disease file, arranged by tumor or tumor site, includes up-to-date information on all neoplasms of current interest. A recently installed terminal is expected to facilitate the updating of these clinical files and the answering of requests. Through an agreement with the National Library of Medicine, the Branch has been provided with on-line access to the more than 1000 recently published journals in the MEDLINE data base. Recurring MEDLARS bibliographies from NLM and citations from DCRT's CBAC tapes aid in the search for promising agents.

During the year the staff continued to be responsible for preparing and distributing the agent folders for Decision Network meetings. Forty folders were prepared for each of 55 drugs. Each folder contained the compound's structure and systematic name, an analog search, a literature search, a summary of clinical data, and the Decision Network questions

and answers. The Section also compiled and distributed the Chemotherapy Program Statistics reports. Data was assembled and cumulated quarterly for the number and types of agents entering the screen, the tests performed, the types of confirmed actives in the primary screen, the compounds passing each Decision Network step, and the drugs for which INDs were filed. Another continuing responsibility is the maintenance of the Chemotherapy Library. Over 2100 journals were circulated and close to $8800.00 was spent on books and journal subscriptions.

II. Contract Operations

The Literature Research Section monitored two service type contracts during the year: One for the preparation of Cancer Chemotherapy Abstracts and the other for microfilming and indexing the chemotherapy literature.

Cancer Chemotherapy Abstracts, a current-awareness serial, provides abstracts of the world's literature on cancer chemotherapy. The new contract for this year provides for the preparation and distribution of CCA on a monthly basis, with the subscription price paying the cost of photocomposition, printing, and mailing by the contractor. As by-products, the Section will receive computer tapes of the abstracts and indexes, and hard copy of all articles cited. Monitoring of the current contract continues to be directed toward improving the quality and timeliness of the service.

The George Washington University project, designed to provide for rapid scanning and easy retrieval of the literature of CCA, was completed. Filmed copies of the 1960-1964 literature, a reader-printer, and cumulated agent and author indexes for this period have been furnished.

PUBLICATIONS SECTION

Program Analysis Branch

The Publications Section is responsible for all publications of the Chemotherapy Program, clearance of contract manuscripts, and editorial assistance to the Chemotherapy staff on a time-available basis.

The Publications Section directs the preparation of Cancer Chemotherapy Reports (CCR), the principal scientific journal developed under the Chemotherapy Program from the original recommendation of the Chemotherapy Committee of the National Advisory Cancer Council. Until 1968, CCR was a single journal but in that year it was expanded to 3 parts. From its inception, CCR (and CCR Part 1) has published original contributions in clinical and experimental studies of cancer chemotherapy, including complete works, preliminary investigations, commentaries and reviews, symposia, and letters pertinent to published articles. All candidate manuscripts are reviewed by 2 or 3 investigators familiar with the subject matter and the final determination of acceptance or rejection is made by the Scientific Editors. CCR is now in its 14th year of continuous publication with a total to date of 91 issues. In 1971, there were 5 issues of CCR Part 1 (Vol. 55, 621 pages).

CCR Part 2, which contains chemotherapy screening data, structure-activity relationships, and other related material, has been published since 1968, as manuscripts and personnel have been available. The most recent issue, Vol. 2, No. 1, was published in late FY71.

There have been 2 issues to date of CCR Part 3, containing non-reviewable program information. The second of these issues, the Proceedings of the First Joint Working Conference, NCI Chemotherapy Program, was published in October 1971. A third issue, containing the 2nd in this series of conferences, is currently in press. It is anticipated that the Proceedings of this conference will be published annually in CCR Part 3. At present, 2 more issues are in the planning stages: a) one containing Protocols for Screening Chemical Agents and Natural Products, and b) one containing clinical brochures, toxicology reports, and other non-reviewable program-related information. These 2 issues are tentatively planned for publication in early FY73.

During the past year, CCR has undergone numerous changes in appearance. A new cover design and color were adopted, as well as changes in the format of each article, the quality and type of paper, and the typeface used. New features were added, such as the inclusion of authors' addresses in the Contents.

CCR has expanded its scope of scientific coverage during the past year, publishing a major review article on acute leukemia and an article resulting from the Joint Clinical Conferences of the NCI's BCRC and the Johns Hopkins University Hospital.

The Publications Section is exploring ways in which to speed up the publication time of CCR Part 1. Although certain absolutes exist in the printing process for all government journals (which must be handled through the GPO), the streamlining of existing editorial procedures could effect noticeable changes in the publication time for this journal.

Comparative data on the CCR series of publications are shown in Table 1. The manuscripts submitted and those that are accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports.

The editorial staffing of the Publications Section has varied over the years, reaching a high of 3 editors in CY67 and CY68 and a low of one editor in the first half of CY69 and again in the last half of CY70 and all of CY71. The Section now has 2 full-time editors.

In addition to the regular journal series, a variety of other publications are edited and prepared by the Publications Section. The Section coordinated the preparation of 2 chemical agent booklets which were distributed at the fall meeting of the ACS in Washington, DC, and the 2nd Joint Working Conference of the NCI Chemotherapy Program in Annapolis, Md. These booklets have received much favorable response with many requests for copies.

The Section prepared, with the support of other PRA sections, an updated and revised version of the Proceedings of the 1st Joint Working Conference, NCI Chemotherapy Program, which was distributed to members of the National Cancer Advisory Board in March 1972.

Another type of special publication prepared by the Publications Section is the "Proceedings of the Chemotherapy Conference on Procarbazine (Matulane: NSC-77213): Development and Application." This conference was given light editorial review and was prepared for printing during FY72.

The Publications Section is also responsible for maintaining the free mailing lists for CCR and Cancer Chemotherapy Abstracts (CCA). CCR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

	3360	Foreign and domestic free mailing (includes ca. 150 in NIH plus storage copies for Section)
	83	GPO files
	500	Superintendent of Documents (for paid subscriptions)
	367	Superintendent of Documents (for Depository Libraries)
Total	4310	

Table 1.--Summary data on the CCR Publications Series

	CY 1967	CY 1968	CY 1969	CY 1970	CY 1971	CY 1972 (Jan 1-Mar 31)
Manuscripts Received	125	107 (Part 1)	78 (Part 1) 4 (Part 2) 6 (Part 3)	122 (Part 1) 5 (Part 2) 1 (Part 3)	107 (Part 1) 6 (Part 2) 11 (Part 3)	25 (Part 1) 0 (Part 2) 11 (Part 3)
Manuscripts Accepted	87	53 (Part 1)	63 (Part 1) 5 (Part 2) 6 (Part 3)	68 (Part 1) 4 (Part 2)	73 (Part 1) 5 (Part 2) 11 (Part 3)	22 (Part 1) 0 (Part 2) 11 (Part 3)
Issues Published	7	7 (Part 1) 2 (Part 2) 1 (Part 3)	6 (Part 1)	6 (Part 1)	5 (Part 1) 1 (Part 2) 1 (Part 3)	1 published, 1 in press (Part 1) 1 in press (Part 3)
Pages Published	561	775 (Part 1) 539 (Part 2) 217 (Part 3)	408 (Part 1)	492 (Part 1)	621 (Part 1) 246 (Part 2) 104 (Part 3)	150 published, 100 in press
Reviewers	143	132	132	140	164	60
Printing Costs[a]	$13,462 ($24/pg)	$22,302 (Part 1) ($28.77/pg) $ 7,877 (Parts 2 & 3) ($10.41/pg)	$11,813.82 ($28.95/pg)	$15,334.51 ($31.16/pg)	$15,000[b] (Part 1) ($24.10/pg) $13,236[c] (Part 2) ($53.76/pg) $2,500[d] (Part 3)	Not available

[a]Costs of GPO contracts with Publications Press, Inc., Baltimore, Md., and Craftsman Press, Inc., Hyattsville, Md.

[b]Estimated costs of 5 issues (final costs currently unavailable).

[c]Actual cost for typesetting ($10,736) and estimated cost for printing and distribution ($2,500).

[d]Estimated cost for printing and distribution only. Costs (estimated or actual) for typesetting not available.

The mailing list for CCA is now being screened to include government employees and members of special advisory groups only, in accordance with a new contract for the preparation, printing, and distribution of this journal.

Another function of the Section is the coordination of clearance of contract manuscripts for publication. In CY71, 161 of these manuscripts were cleared, and during the first quarter of CY72, 32 manuscripts were cleared. In assuming the responsibility for clearance of these manuscripts, the Publications Section checks all NSC numbers mentioned in the papers, obtaining publication clearance from suppliers for those compounds that have not previously received such clearance. The authors are instructed to reword footnotes as necessary to give proper credit to the Chemotherapy Program. The Section ensures that the Project Officer sees all papers and signs the correct forms. Records and reprints are kept for all manuscripts cleared, and quarterly reports are prepared for distribution to staff personnel.

SCIENTIFIC RECORDS SECTION

Program Analysis Branch

The Scientific Records Section (SRS) provides supporting services and information to members of the Chemotherapy staff in nearly every phase of the development of chemotherapeutic drugs.

The Section prepares and maintains the master structure file and several cross-reference files on each drug. It indexes and organizes new compounds into files suitable for retrieval by name, NSC number, molecular formula, and chemical substructure. Section personnel also prepare shipping lists for the transmittal of compounds to screeners and coordinate the various special shipping instructions from DR&D for certain categories of agents.

As biological data are returned by screeners, section personnel monitor some aspects of its quality and verify its entry into computer files. Screening data for both natural products and synthetic compounds are sorted, checked, and filed, and requests for copies of specific data are filled from film or paper, or by computer inquiries. The production of several monthly status reports on active synthetic and natural products is coordinated by Section personnel.

In order to fully utilize the files of chemical and biological data that have been built in the past 17 years, the Section has the ability to search the chemical file by structure or substructure and provides data such as structural formula, systematic name, and biological data in a format useful to requesters. All data for structure-activity studies are gathered in this Section with the cooperation of other sections in PRA, and new methods are investigated to facilitate such correlation.

Chemical File

A. Registry of New Compounds

The structures of all compounds received in the Drug Development Branch (DDB) are sent to Chemical Abstracts Service (CAS) for computer determination of their newness to the chemotherapy file. After structure cards have been returned to NCI, new compounds are named and assigned NSC numbers, and the supplier is notified of the numbers assigned to his compounds. The compounds are then shipped to the appropriate screener.

In anticipation of an increased number of synthetic compounds in an accelerated program, the Section, in cooperation with DDB, made two trial changes in procedures.

1. Flexowriter tapes of nomenclature information are no longer produced, and the finished chemistry card was discontinued in favor of the 'rough' chemistry card. As a corollary to these changes, SRS produced Xerox copies of supplier submission sheets with NSC numbers stamped on them for return to suppliers.

2. Mrs. Mary Flather of DDB was detailed to SRS on a trial basis to consolidate structure and naming functions in PRA, and thus allow DDB to concentrate on acquisition. As part of this experiment, an attempt will be made to see where clerical assistance can supplement the naming and structuring operations.

The scheduling of computer registry runs at CAS was changed from biweekly to weekly during the year to allow more current data. Personnel in PRA and DDB responsible for chemical information became more familiar with the registry systems-flow through a site visit to CAS and an analysis of the timing data for selected registry runs early in 1972. This additional exposure to the details of the computer system was valuable in monitoring the routine NCI registry procedures, and in addition provided helpful experience for the new registry systems being planned.

B. Shipping

The Section shipped 10,151 compounds to screeners in 1972 and maintained the master screener and supplier address file used in the transmittal of biological data. With the elimination of new flexowriter tapes, the transmittal of chemical names to screeners was discontinued.

A procedure was initiated in which screening data and chemistry cards for all duplicates were sent to the Drug Evaluation Branch (DEB) for evaluation of testing adequacies in current protocols. In a majority of cases, shipment for rescreening was initiated by DEB.

C. Name and Formula Indexes

Systematic names and synonyms, plus supplier name and discreet and publication codes, are stored in a text file at CAS. Quarterly indexes sorted by molecular formula and alphabetically by name are computer-produced. To improve the quality and usefulness of this file, three steps were taken during the year.

(1) With DDB cooperation all names were reviewed and extraneous ones deleted.

(2) CAS registry numbers and synonyms for active compounds were obtained by computer methods where available and added to the file.

(3) Equipment was obtained for easier access to these indexes.

D. Card File Microfilming

In April 1972, the Section completed a large-scale microfilming of all chemistry card files. After the film has been inspected, all redundant card files will be destroyed, and only one master card file will be maintained and physically updated. The master film will be maintained at the Federal Records Center in Suitland, and copies will be available for

viewing in SRS. Approximately 500,000 chemistry cards were filmed plus 110,000 archival records of the previous manual retrieval methods.

E. Chemical File Analysis

The Section maintains the master file of codes used for individual suppliers and cooperated during the year in the conversion of this punched card file into a direct access system at DCRT.

To more effectively utilize the supplier code file, all organizations supplying compounds were assigned codes designating their status as government, commercial, or university/non-profit organization, with a further code to designate foreign organizations. A computer program is being written to analyze the biological file by supplier, listing all NSC numbers for each supplier, and showing the total number of compounds submitted per year since 1956 by each supplier.

The results of these experimental analyses will be evaluated and made available to DDB as an aid to acquisitions.

In a cooperative effort with the DDB, the Data Processing Section (DPS), and the Literature Research Section, the SRS continued its investigation of magnetic tape services of secondary scientific information services such as CAS and ISI. All tape services of possible interest and the information centers providing them were summarized for the Chief, PRA, by SRS, and experiments were run to determine their usefulness in (a) monitoring the literature for agents active against cancer, and (b) providing leads to sources of classes of compounds of interest to the Chemotherapy Program.

Biological File

A. Data Input

The increase in screening during the year resulted in a significant increase in the workload for those members of the Section responsible for:

a) checking control packs and control pack indexes;

b) sorting and routing natural product data, and filing both natural product and synthetic data;

c) researching computer error outputs and correcting the master data files.

The large increase in the number of lines of data processed in 1971, compared to 1970, combined with a change back to weekly processing, resulted in a large increase in the amount of paper handled by Section personnel.

B. File Maintenance and Inquiries

The microfilming of the entire biological file by PRA resulted in less crowded paper files and more convenience in retrieving data. With weekly processing, computer inquiry was used more often to answer requests for screening data rather than Xeroxing or printing from microfilm. Queries of the magnetic tape file for compounds, and sometimes data, for individual suppliers was used routinely as part of the DDB analysis of research grant proposals.

C. Monitoring of Compounds of Interest

Section personnel continued to prepare input and check output for information used in monitoring active agents and those compounds for which more compound was needed for testing. Lists, such as the Material of Interest (MOI), the Confirmed Active Plants (CPAM), the Selected Agents List (SAL), and the Quantity Not Sufficient (QNS) (available on request), serve as reference tools in evaluating natural products, active synthetics, and agents requiring an additional supply. They are also used as reference documents by screeners and other Chemotherapy investigators.

D. Systems Testing and Evaluation

The three Section personnel involved in the biological data processing system took an active part in testing the procedural efficacy of portions of the biological data processing system. Exception reports normally handled by the data processing contractor were researched and corrections made to determine any improvements that might be made in procedures. Control packs for one screener were edited comprehensively in a limited trial for a similar reason.

Chemical-Biological Interaction

Because of its responsibility for maintaining both the chemical and biological files, the Section plays a pivotal role in all studies of structure-activity relationships.

A. Substructure Search

The ability to search all chemicals in the file for selected fragments (such as purine rings) is the most useful tool in structure-activity studies. Search is used in structure-function studies by the Drug Evaluation Committee (DEC) and at Decision Network 2 of the linear array. In addition, it is available as a retrieval tool for interested Chemotherapy scientists and, on a more limited basis, to qualified outside requestors. During the year, the Section continued to use the CAS substructure search system located at CAS, and in addition supervised the installation of parts of the system used by NCI at the NIH computer division.

B. Searches Run

One hundred seventeen searches of the master file were run during the year plus 17 updates of previous searches of interest. These searches covered a wide spectrum of interest, and included support for large structure-function studies of the 1,4-quinones, the nucleosides, and the folic acid antagonists.

Questions were formulated and coded by SRS personnel and checked at CAS before searching there. Output was provided to users as lists of NSC numbers, copies of structures, and biological data where requested.

In March 1972, the Search Unit in SRS was reorganized to allow quicker, more flexible response to search queries, and to more efficiently use the search capability now available at DCRT. This reorganization, together with more rapid scheduling of searches at CAS, should give faster turn-around of searches in the coming year.

C. Advances in Search

The Section continued cooperating with the NIH computer division on an interactive search system for the PDP-10 computer. The results of all CAS searches for Chemotherapy, plus SRS analyses of these results, were given to the University of Pennsylvania to aid in the design of a new Chemotherapy Chemical Information System. Representatives of the University of Pennsylvania, SRS, and DPS met several times during the year to discuss possible cooperation in the search module of the Chemical Information System's design.

Two efforts were made during the year to systematize and shorten the procedures used in structure-activity studies.

1. A procedure was developed and tested in which summarized biological data for 1600 1,4-quinones would be entered together with chemical fragment data into a computer-searchable file. This will eliminate the handling of paper in the final evaluation stages of the project, and in addition allow easy retrieval of data for any conference to discuss this class of compounds.

2. Using the experience with 1,4-quinones, representatives of DDB and PRA discussed methods to create computer subfiles of chemical and biological data at the beginning of analysis and to use these files as a supplement or even replacement for the paper files used in the past. Answers have been retrieved in machine-readable form from the chemistry file and will be used directly to retrieve the corresponding data.

The experience with these large-scale studies points out the need for close cooperation from the beginning of all large structure-activity studies between chemical and biological file experts, chemists and biologists (for analysis), and even representatives of such journals as Cancer Chemotherapy Reports to determine proper outputs of the study.

This multidisciplinary approach, together with improvements in search technology and coordination of files, will greatly reduce the time and effort needed for future structure-activity correlation.

Seminars, Training, and Awards

As part of the CAS contract, a two-day seminar in the CAS computer registry and substructure search system was conducted for interested Chemotherapy staff members. The seminars gave a good overview of the interrelation of the computer systems and the uses to which the computer is put in handling chemical information.

Miss Barbara Quarles, a stay-in-school employee in SRS, was given a cash award based on our recommendation.

All Section personnel have now taken at least one data processing course, and most have taken Wylbur. One person in the Section attended the Upward Mobility College, and the two chemists attended night school toward degrees at American and George Washington Universities.

Contracts Monitored

A. Chemical Abstracts Service (PH43-65-1021)

Computer files are maintained at CAS to duplicate-check new input, perform substructure search, and provide name and formula indexes. The files now contain over 170,000 structures and 270,000 names and synonyms.

B. University of Pennsylvania (NIH-71-2187)

The Section primarily gave consultation in the area of substructure search, providing analysis of past questions and user habits, and critiquing possible future search system designs.

SUMMARY REPORT

EXPERIMENTAL THERAPEUTICS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 - June 30, 1972

The Experimental Therapeutics area studies many facets of the interaction between drugs and biological systems. It includes biochemical pharmacology, toxicology, physiological disposition, drug metabolism, molecular biology, mechanism of action, and comparative pharmacology. In addition, it works closely with the Clinical Trials area with regard to clinical research problems.

Organizationally, Experimental Therapeutics consists of the Office of the Associate Scientific Director and four discrete but closely coordinated laboratory research units:

The Office of the Associate Scientific Director, in addition to an overall administrative responsibility for four laboratories, conducts a research effort in the area of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid. The studies are particularly aimed at improving our ability of getting drugs into tumors within the central nervous system either through the elucidation of structural requirements necessary for a molecule traverse the "barrier' or through the design of new delivery techniques. Another important area of research concerns biochemical pharmacology of antitumor drugs in preclinical systems, an effort which blends well with ongoing activities in the laboratories within the Experimental Therapeutics area.

The Laboratory of Toxicology, whose primary responsibility is to investigate and describe the qualitative and quantitative toxicity of potential anti-neoplastic drugs using animal test systems. Attempts are made to define the predictability, reversibility, and mechanism of drug-induced toxicity. These functions of the laboratory are closely integrated with the preclinical development of new anticancer agents and ongoing clinical pharmacologic and toxicologic studies. An equally important responsibility of the laboratory is to conduct basic research directed towards a thorough understanding of the toxicity of anticancer agents. In addition, the laboratory and its senior investigators share the administrative responsibility for routine and special preclinical toxicologic studies performed on contract with private laboratories or universities;

The Laboratory of Chemical Pharmacology, whose purpose is to study and elucidate the qualitative and quantitative aspects of the physiological disposition of antitumor agents in man, laboratory animals and in vitro

predictive systems and to conduct basic research on general principles of pharmacology. To achieve the latter major emphasis is placed on the development and use of analytical techniques for the measurement of antitumor drugs and their metabolites in biologic media.

Considerable effort is also directed towards the development and use of mathematical pharmacokinetic models for predicting drug distribution in normal and tumor tissue from one animal species to another and finally to man. The objective is to permit the design of more effective therapeutic doses and schedules for the clinic. The elucidation of the mechanism of action of antitumor drugs is also pursued and metabolites of antitumor drugs are isolated and tested for antitumor activity and cell cycle effects. Structure activity relationships are conducted on analogs of antitumor agents being investigated. Studies are performed on non-microsomal and microsomal mammalian enzyme systems which metabolize drugs and the identification of factors which alter the activity of these enzymes and may influence the activity or toxicity of various antitumor agents. The laboratory is also responsible for the administration and supervision of studies performed in contract with extramural organizations. The contracts are primarily concerned with the development of analytical-biochemical methodology and the study of pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and in vitro predictive systems. Approximately 12 agents are studied each year which are of interest to the Chemotherapy Program;

The Laboratory of Molecular Pharmacology, whose function is to investigate mechanisms of drug action at the molecular level through physical chemical studies on the interactions of drugs with nucleic acids (DNA, RNA) and their effects on nuclear protein metabolism in intact mammalian cells and in in vitro systems. This work aims toward an understanding of the molecular basis for the biological effect of antitumor drugs;

The Laboratory of Tumor Cell Biology, whose function is to conduct studies on: (a) the physiological, cytogenic and biochemical control mechanisms in normal and neoplastic cells; (b) development of biological or biochemical markers for the presence of small numbers of neoplastic cells; (c) purification and biochemical characterization of DNA polymerases from human cells and from oncogenic RNA viruses ("reverse transcriptase" or RNA-dependent DNA polymerase) The latter studies are aimed at understanding the function of these enzymes with respect to appearance in the cell cycle, activity with certain c mopunds, role in cellular differentiation and neoplastic transformation. As a logical extension of the above objectives the laboratory and its senior investigators are responsible for the administration and scientific evaluation of several studies performed on contracts with private laboratories, universities, and other government agencies.

Areas of significant and exciting findings reported by investigators of the various laboratories within Experimental Therapeutics are summarized below:

Office of the Associate Scientific Director

1. Development of a model for studying penetration of antitumor drugs into the central nervous system or into neoplastic tissue within the brain following intravascular or intrathecal routes of administration.

2. The development of 5-methyl-d,l,tetrahydrohomofolate as a potential antitumor drug for treatment of human malignancy and the elucidation of its biochemical pharmacology.

Laboratory of Toxicology

1. Correlation between quantitative toxicity of antitumor agents in preclinical species (dogs and monkeys) and man.

2. Biochemical toxicology of L-asparaginase.

3. Pathogenesis of drug induced diseases.

4. Effect of antitumor agents on reproduction.

Laboratory of Chemical Pharmacology

1. Pharmacologic disposition of antitumor agents in man and animals including methotrexate, guanazole, MeCCNU, camptothecin and gallium^{67}.

2. Development and refinement of a pharmacokinetic model primarily utilizing methotrexate.

3. Correlation of *in vivo* and *in vitro* pharmacokinetic parameters with biologic effects of antitumor drugs.

4. Evaluation of usefulness of several induced primary and transplanted tumor systems as potential screens. Particular attention was given to certain antiviral compounds including Poly rI; Poly rC, interferon, tilorone hydrochloride, rifamycin SV, rifampicin and derivatives, and amantadine derivatives. In addition, hydroxyguanidine, hycanthone, and salts of the Group IIIa metals of aluminum, gallium, indium and thallium were also studied.

5. Studies of relative significance of microsomal, mitochondrial, and soluble supernatant enzyme systems in the metabolism of antitumor agents.

6. Studies in monkeys: Availability of a large breeding and experimental monkey colony (600 animals representing 5 species) permits a variety of studies of antitumor agents. Induction of hepatoma has provided an experimental system for the evaluation of potential chemotherapeutic agents and for studies of various fundamental aspects of this tumor including α-fetoprotein production and its use as a marker. Of particular significance was the induction of malignant lymphomas and lymphocytic leukemia in owl monkeys inoculated with *Herpes saimiri* virus.

Laboratory of Molecular Pharmacology

1. Interaction of anthramycin with DNA: Anthramycin was found to constitute a new type of chemical structure that reacts covalently with DNA. The effects on the hydrodynamic properties and light scattering of DNA indicated a previously unreported type of effect on DNA conformation.

2. Effect of drugs on RNA metabolism in L1210 cell culture: These studies indicate that: (a) drugs that react covalently with DNA reduce the size of RNA molecules synthesized but do not selectively, inhibit nucleolar 45s RNA synthesis or processing; (b) interchalating agents tend to increase the size of the nucleoplasmic RNA molecules synthesized and interfere with the processing of nucleolar 45s RNA; and (c) selective inhibition of nucleolar 45s RNA synthesis appears to be characteristic of agents that bind tightly but not covalently to DNA and that have a delayed dissociation from DNA. The nitrosoureas and camptothecin were focused upon in the above studies.

Laboratory of Tumor Cell Biology

1. Studies on tRNA methylation by tumor cells: Methotrexate, of a variety of antitumor drugs studied, produced the most significant inhibition of tRNA methylation in L1210 cells grown in tissue culture. In monkeys, tRNA methylase activity increased in fetal liver, hepatomas and pre-malignant liver, the latter correlating with serum α-fetoprotein. Evidence was found for the presence of a tRNA methylase inhibitor in normal liver but absent in fetal liver or hepatoma.

2. Polymerases, RNA dependent (reverse transcriptase) and DNA dependent:

a) In body and fluids: Serum and plasma from leukemic patients and from patients with other types of tumor did not show evidence for reverse transcriptase. However, DNA polymerase activity was found in some sera from patients with acute leukemia.

b) In cells: The laboratory did demonstrate the presence of reverse transcriptase in leukemic cells and was the first to report in any cell that a DNA polymerase may make DNA from RNA. Reverse transcriptase in various RNA tumor viruses was also purified and evidence indicated that the enzyme is required for replication of RNA tumor viruses and transformation by these viruses. A number of rifampicin derivatives have been uniquely shown to selectively destroy human leukemic cells with no effects on normal white cells. These agents were also shown to inhibit DNA polymerases of leukemic cells and the enzyme of tumor viruses.

Purified antibodies to viral reverse transcriptase (Moloney sarcoma virus and feline leukemia) are currently being developed for studies to determine the specific presence and cross-reactivity of reverse transcriptase in a variety of biologic samples.

3. Maturation studies: A reliable method was developed for the identification of myeloid stem cells in acute leukemia utilizing pulse labelling with ^{3}H-thymidine in peripheral blood to study maturation of blasts. Patients with CML showed cell precursors in blastic transformation that have a diminished capability to mature despite the early presence of mature neutrophils *in vitro*. The *in vitro* infection of fresh leukocytes with Epstein-Barr virus resulted in blastoid transformation of lymphocytes and infinite growth potential; this transformation was accompanied by major chromosome changes.

4. Cytogenetic studies: It was shown that a chromosomal marker (such as sex chromosome, etc.) could be used as an indicator for bone marrow transplantation. The C marker chromosomal aberration was also clarified; it is not a true secondary constriction but rather a random gap or break that could have been caused by a non-specific viral infection. Chromosomal analysis of L-asparaginase treated rabbit mothers and fetuses showed normal karyotypes. L-asparaginase decreased the mitotic index of rabbit blood cells in culture.

5. Biologic and biochemical markers: A plan for an approach to a biologic-biochemical marker program was initiated and several extramural contracts were awarded to support this phase of the laboratory's research responsibility. The need for "markers" for the quantitative measurement of residual tumor cells after therapy is a critical problem in the management of patients with cancer.

ANNUAL REPORT

OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 -- June 30, 1972

Studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in normal and experimentally disturbed animals have been undertaken. Intravascular infusions and ventriculocisternal perfusions have been the methods generally employed. Determinations of brain capillary and cellular permeability to a variety of physiological materials (inorganic ions, amino acids, and monosaccharides) and drugs have been made in normal monkeys and dogs. These studies indicate that the brain capillaries are permeable to very small polar materials (MW<100) and most non-polar compounds and that these features of blood-brain exchange resembles those of cellular membrane exchange. With ventriculocisternal perfusion experiments we have determined the distribution of specific anti-neoplastic agents (e.g. hydroxy-urea) from CSF to brain parenchyma and the limitations of intrathecal (into the CSF) injections and infusions for delivering drugs to central nervous system (CNS) lesions. If an adequate brain tumor model becomes available, similar studies of blood-tumor and CSF-tumor transport will be initiated using those same experimental approaches. A critical evaluation of the effectiveness of intracarotid infusions in delivering drugs to the CNS is currently underway. This study involves experimental work in normal monkeys with a series of pharmacological compounds and development of a computer model for describing the distribution kinetics of this system. The interactions of ions such as sodium and chloride and of drugs such as ouabain in the formation, flow, and absorption of CSF are being examined in normal, hydrocephalic, hyper- and hypovitaminic A monkeys. It has been found that the rate of CSF production and the size of the brain extracellular space can be changed by the intraventricular infusion of various drugs and that the flow and absorption of CSF is affected by Vitamin A. From work on elasmobranchs, we have demonstrated a definite dependency of net brain uptake upon blood-CSF exchange. Certain materials enter the brain tissue from the blood but are removed to an extent by drainage into the CSF; whereas other compounds rapidly enter the CSF from the blood and, in turn, move from CSF into brain parenchyma. In other studies on these animals, we have examined the distribution kinetics of a series of polar materials in a variety of tissues (heart, skeletal muscle, liver, etc.) and compared them to mammalian values.

Serial No. NCI-3500
1. Office of the Associate Scientific Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Distribution of Drugs between Blood, Brain, and Cerebrospinal Fluid*

Previous Serial Number: Same

Principal Investigators: Joseph D. Fenstermacher

Other Investigators: Ronald B. Blasberg, Walter Eckman, Alan Cowles, Clifford S. Patlak, and David P. Rall

Cooperating Units: Biometrics Branch, NIMH; Office of the Director, NIEHS

Man Years

Total:	6.5
Professional:	4.5
Other:	2.0

Project Description

Objectives:

The objective of this project is to quantify the transport of various materials of physiological and pharmacological importance between blood, brain, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to aid the development of chemical methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The methods of ventriculocisternal and ventricle-ventricle perfusion of various radioactive materials has been utilized in monkeys and dogs. In addition studies of brain and CSF uptake and distribution of intravenously infused, radioactively labeled physiological and pharmacological materials have been examined in dogs, monkeys, and elasmobranchs. An experimental system employing simultaneous intracarotid and intravenous infusion has been developed and used. For the CSF absorption studies a constant pressure, flow-monitoring system has been employed.

Major Findings:

From our ventriculocisternal perfusion studies, we have measured the ependymal, pia-glial, cellular, and capillary permeability of the central nervous system to a variety of physiological and pharmacological materials. The brain-CSF interfaces (the ependyma and pia-glial border) are highly permeable to polar compounds of 5000 MW and smaller and fairly permeable to polar compounds having molecular weights between 5000 and 70000, thus these membranes only limit the CSF to brain exchange of very large compounds. Significant brain cell uptake of several physiological (e.g. α-amino-isobutyric acid and cycloleucine) and pharmacological (e.g. hydroxy urea and cytosine arabinoside) compounds was noted. The exchange of material between blood and brain was found to resemble that between extracellular and intracellular fluid, suggesting that the blood-brain barrier is a cellular-type membrane. Using the same experimental approach, we have also determined that materials can diffuse through the extracellular fluid of the brain nearly as rapidly as through water.

Our work on the distribution of four antitumor agents - methotrexate, hydroxy-urea, thio-tepa, and cytosine arabinoside - into brain tissue during ventricular perfusion has strong implication about the efficacy of intrathecal drug injections and infusions. The two lipid soluble drugs in this study, thio-tepa and cytosine arabinoside, did not penetrate very deeply into brain tissue (<1.0 mm) because of their rapid exchange across brain capillaries and loss into the circulating blood. The two polar drugs, methotrexate and hydroxy-urea, did not "leak" appreciably across the brain capillaries; their depth of penetration was limited by their respective diffusibilities in brain extracellular fluid and the duration of perfusion. After four hours of perfusion significant levels of both drugs could be found from 3-4 mm in from the perfused surface; however much longer periods of perfusion (8-24 hours) appeared to be necessary to get much deeper penetration. This work strongly suggests two things: 1) that the intrathecal administration of lipid soluble drugs would not lead to significant accumulation in brain tissue and would be of little value in brain tumor chemotherapy and 2) that the intrathecal administration of polar drugs, if infused continuously over several hours time, would provide adequate amounts of drug to sites near the brain surface (or CSF) but not to deeper tissue.

The experimental analysis and computer modeling of intracarotid infusions suggest, at present, that this route of administration does not greatly enhance the distribution or accumulation of most drugs within the CNS. Tentatively we have concluded that intracarotid infusions are superior to intravascular infusions only for a non-polar drug which is quickly and tightly bound to active sites within the brain tissue or is rapidly metabolized within the brain to a polar, active compound.

The design and testing of drug distribution computer models has been undertaken in attempt to develop some general principles for brain tumor chemotherapy. Three different routes of administration - intravenous, intracarotid, and intrathecal - have been considered. By varying the

physico-chemical and biological properties of the drug and the infusion site, we have obtained theoretical guidelines for brain tumor chemotherapy. At present we are rigorously testing the design of the model and only have preliminary data; experimental verification of the predicted general principles will be attempted.

We have continued our studies in the comparative physiology and pharmacology of blood-brain-CSF transport. Marked differences in the transport of polar compounds between blood and brain and blood and CSF have been found in elasmobranchs. The brain and/or choroid plexus of the hag fish (Myxine glutinosa) and the nurse shark (Ginglymostoma cirratum) are considerably more permeable to inulin and sucrose than are those of the spiny dogfish (Squalus acanthias), the sting ray (Dasyatis sp.), and the common small skate (Raja erinacea). The composition and turnover of cerebrospinal fluid and pericardial fluid in the spiny dogfish have been studied. Each of these fluids has a unique and specific composition and appears to be produced by a secretory process rather than filtration. Carbonic anhydrase inhibitors were found to significantly alter the volume and turnover of CSF in these animals. Further studies of the distribution kinetics of polar materials in other body tissues, e.g. liver, heart, and skeletal muscle, have shown that the sizes of the vascular, extracellular, and water space in these tissues are generally similar to those measured in mammalian species but that the rates of capillary exchange and, especially, cellular uptake are slower in elasmobranchs than in mammals. This work was done at the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine; and the Lerner Marine Laboratory, Bimini, The Bahamas.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the central nervous system. Little information is available concerning the penetration of antitumor drugs into the CNS or into neoplastic tissue within the brain. Basic research on this aspect of the pharmacology of antitumor drugs is necessary for the rational use of these agents. Furthermore, it is becoming clear that the capillary endothelium of central nervous system (the presumed site of the blood-brain barrier) acts in many ways as a typical cellular-type membrane and the entry of drugs into the central nervous system parallels the entry of drugs into cells in general. This knowledge of the characteristics of brain transcapillary exchange coupled with our understanding of CSF-brain exchange will enable the chemotherapeutist to choose the best route (intravascular or intrathecal) of drug administration to CNS lesions.

Special transport systems appear to exist for moving various necessary material, e.g. amino acids, from blood to brain and CSF in normal animals and for moving various end products of metabolism, e.g. weak organic acids, from the brain and CSF. It is necessary to study and understand these mechanisms and quantitate them for normal brain and CSF. Similar studies of brain tumors and other pathological brain and CSF conditions should also be

made in an attempt to examine the physiology, biochemistry, and metabolism of these diseases. The type of work performed in our laboratory on this subject is important and meaningful for an understanding of the biology of brain and meningeal tumors.

Proposed Course:

1. Continue our studies of the transport of various physiological (e.g. amino acids and simple sugars) and pharmacological (e.g. the nitrosoureas) materials between blood, brain, and CSF in normal animals, and more precisely quantitate the permeability of brain capillary and cellular membranes.

2. Seek an adequate primary brain tumor model to use for physiological and pharmacological studies similar to those previously done on normal animals.

3. Carefully examine the brain uptake of drugs when infused intracarotidly and evaluate the effectiveness of this route of administration of chemotherapeutic agents.

4. Continue to examine the mechanisms and sites of CSF secretion and the effects of various drugs and pathological conditions on this process.

5. Screen a series of nitrosourea compounds for effectiveness against the intracerebrally transplanted murine ependymoblastoma.

6. Continue to study the mechanism, sites, and capacity of CSF absorption in normal and hyper- and hypovitaminic A animals.

7. Finish the design and testing of our computer model for drug distribution to brain tissue (both normal and neoplastic) and generate the necessary supporting experimental data.

8. Investigate further the comparative physiology and pharmacology of material transport between blood, tissue, and fluids in lower vertebrates since these animals offer certain anatomical and physiological advantages for the study of fluid formation and material exchange.

Publications:

1. Fenstermacher, J. D. and Rall, D. P.: The physiology and pharmacology of cerebrospinal fluid. In Carpi, A. (Ed.): International Encylopedia of Pharmacology. Section 33; (Cerebral Circulation). London, Pergamon Press, 1972 (in press).

2. Fenstermacher, J. D.: Ventriculocisternal perfusion as a technique of studying transport and metabolism within the brain. In Marks, N., and Rodnight, R. (Eds): Methods in Neurochemistry. New York, Plenum Publishing Corp., 1972 (in press).

3. Rall, D. P. and Fenstermacher, J. D.: Volume of cerebral extracellular fluids. In Siesjo, B. K., and Sorensen, S. C. (Eds): Alfred Benzon Symposium III, Ion Homeostasis of the Brain. Copenhagen, Munksgaard, 1971, pp. 29-37.

4. Milhorat, T., Hammock, M. K., Fenstermacher, J. D., Rall, D. P., and Levin, V. A.: Cerebrospinal fluid production by the choroid plexus and brain. Science 173: 330-332, 1971.

5. Cserr, H. F., Fenstermacher, J. D., and Rall, D. P.: Brain-barrier systems in sharks. J. Comp. Biochem. Physiol., 1972 (in press).

6. Fenstermacher, J. D. Sheldon, F., Ratner, J., and Roomet, A.: The blood tissue distribution of various polar materials in dogfish, Squalus acanthias. J. Comp. Biochem. Physiol., 1972 (in press).

* Annual Report previously entitled, Penetration of Antitumor Drugs into the Central Nervous System.

Serial No. NCI-3516
1. Office of Assoc. Sci. Dir. for Exp. Ther.
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: Same

Principal Investigator: J.A.R. Mead

Other Investigators: A. E. Auletta, C. T. Hardesty, L. C. Mishra and R. Tomchick, Microbiological Associates, Inc.; A. Goldin, H. B. Wood, Jr., J. Driscoll, A. W. Schrecker and R. H. Adamson, NCI; J. J. Coffey, Arthur D. Little, Inc.; M. Friedkin, University of California, San Diego; R. L. Kisliuk, Tufts University School of Medicine: W. R. Jondorf, George Washington University School of Medicine; A. Taunton-Rigby and O. M. Friedman, Collaborative Research, Inc.

Cooperating Units: None

Man Years

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

To investigate the biochemical pharmacology and biochemical toxicology of antitumor agents in experimental preclinical systems to better define the parameters which influence the chemotherapeutic activity of new or established agents.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

The studies in this project can be categorized under the following headings:

A. Folic Acid Antagonists
B. Homofolate Derivatives
C. 5-hydroxypicolinaldehyde thiosemicarbazone
D. Ellipticine and its Derivatives
E. Cyclocytidine
F. Dihydrofolate Reductase Radiometric Assay in vivo
G. Emetine and Other Antiparasitic Compounds

A. Folic Acid Antagonists

Since last year's annual report was written several more, newly synthesized, analogs of methotrexate have been evaluated for their antileukemic potency in the advanced L1210 system relative to methotrexate. The purpose of these studies was to increase our knowledge of the impact structural changes make on the biological effectiveness of methotrexate. The results were of interest from several points of view in that not one of the new compounds had antileukemic activity significantly superior to methotrexate. This points out very clearly the developmental hazards attendant on molecular manipulation is the absence of clearly defined structural requirements for optimizing biological activity. All of these derivatives were able to inhibit dihydrofolate reductase at approximately the same concentration. The dose levels in general were of the same order as has been previously observed. Monosubstituted derivatives had optimum doses comparable to methotrexate whereas disubstituted derivatives had dose levels similar to those of dichloromethotrexate. There were, however, some interesting exceptions to this general observation namely that the dose levels of 2'-chloromethotrexate and 3'-methoxymethotrexate were more like those of the disubstituted derivatives. It will be of considerable interest to determine whether this reduced potency per mg is due to extensive metabolic transformation of the drugs as it may point the way to development of methotrexate derivatives with heightened selectivity for man. At present it is unfortunately true to say that none of the methotrexate derivatives examined in these studies would merit a clinical trial based solely on their in vivo activity against advanced subcutaneous leukemia L1210. The lack of sound scientific basis on which to rationally select one of two agents from a group of active analogs is becoming a major problem for the Chemotherapy program e.g. there are presently over 50 L1210-active alkylating agents in limbo because there is no rational way to justify taking them further along the path towards clinical trial. The series of methotrexate derivatives described above will now be systematically examined in a series of tests to see whether any one of them has a unique quality which could justify their further development. Minor differences in antitumor effectiveness are obviously not sufficient to do this so, metabolic susceptibility, protein binding, duration of effect, uptake and tissue penetratability will be used to explore the potentialities of each for selection purposes.

In another series of experiments the biological activity of N_5-acetyltetrahydrofolate has been examined. This is the next higher homolog of 5-formyltetrahydrofolate (citrovorum factor) and like citrovorum factor is chemically quite stable and resistant to oxidation. In view of the observed antileukemic activity of tetrahydrohomofolate and related compounds, the antileukemic

activity of 5-acetyltetrahydrofolate against the strongly antifolate-resistant tumor, L1210-FR8, was determined. It was completely inactive on the QD, day 1-9 schedule at doses up to 600mg/kg/day which were also without visible manifestation of toxicity. The next question was whether the compound could act as a source of tetrahydrofolate and reverse the antileukemic effect of methotrexate. In a comparative experiment with citrovorum factor it was found to be without effect on the activity of methotrexate against leukemia L1210 whereas citrovorum factor at equivalent doses completely reversed the methotrexate effect. This lack of biological effect of 5-acetyltetrahydrofolate may point the way towards a selective release of tetrahydrofolate in certain tissues, in view of the present interest in high-dose methotrexate: citrovorum factor rescue therapy. Thus if a 5-substituted derivative of tetrahydrofolate could be found which released tetrahydrofolate selectively in host tissue but not tumor tissue, the antitumor effect of methotrexate on high-dose therapeutic regimens could be considerably heightened.

B. Homofolate Derivatives

Further work has been carried out on the pharmacological activity of this series of compounds. The principal findings during the past year have centered on 5-methyl-d,l,tetrahydrohomofolate. The initial findings that this compound was equally as active against the antifolate-resistant L1210-FR8 as tetrahydrohomofolate has been confirmed as has the observation that it is significantly active against the parent L1210. Schedule dependency studies have shown that the optimal treatment schedule is daily until death and that when the treatment is commenced as early as day 1 after leukemic inoculation the optimal dose appears to drop from 200-400mg/kg/day to 50mg/kg/day when treatment is continued until the death of the animals. The reason for this is not immediately apparent as the higher dose levels are well tolerated giving no indication of toxicity. This aspect of the agents therapeutic effect is being investigated further.

Perhaps the most intriguing aspect of the agents action is that it is a very weak inhibitor of L. casei thymidylate synthetase. This is in sharp contrast to the strong inhibitory effect of tetrahydrohomofolate on the same enzyme. This observation probably indicates that 5-methyltetrahydrohomofolate is not acting as a thymidylate synthetase inhibitor in vivo. The question then arises as to how it is exerting its rather specific and selective differential antitumor effect. An attractive speculation is that it is in some way interfering with methionine biosynthesis and that the greater sensitivity of L1210/FR-8 to it reflects a greater dependence of the antifolate-resistant tumor on this pathway. This hypothesis is currently being investigated. Alternatively, the observation by Borek that t-RNA methylases, which are observed in relatively high concentrations in a majority of tumors but not normal tissue, can be inhibited by S-adenosylhomocysteine raises the interesting possibility that by competing with 5-methyltetrahydrofolate, 5-methyltetrahydrofolate prevents t-RNA methylation and thus interferes with tumor growth. This possibility will also be investigated. At present it would appear that 5-methyltetrahydrohomofolate is an agent of considerable potential and it will be vigorously examined from a variety of standpoints in the

months ahead.

In a study on the metabolism of dihydrohomofolate in monkeys it was found that it was readily converted in vivo in the liver to tetrahydrohomofolate. Since the monkey has dihydrofolate reductase levels comparable to man this finding suggests that dihydrohomofolate could be a useful agent in man if need be.

Previous studies have shown that a single injection of either folate or homofolate inhibits the dihydrofolate reductase in homogenates prepared from kidneys of normal mice. Furthermore, the enzyme activity of such homogenates can be restored by removal of these agents from the preparation by diafiltration. It has been suggested that the enzyme inhibition which is seen in vitro is due to the precipitation of folate and homofolate in the kidneys of treated animals. This renal damage also causes an increase in DNA synthesis. Stimulation of the uptake of ^{3}H-thymidine occurs from 30 to 72 hours after drug administration. In an attempt to elucidate the biochemical events which occur in vivo following drug administration this study has been expanded to include new agents and the uptake of radioactive precursors of DNA other than ^{3}H-thymidine. (In addition) in vivo activity of kidney dihydrofolate reductase will be assessed according to the method of Mishra et al.

The uptake of ^{3}H-deoxycytidine and ^{3}H-deoxyuridine follow the same pattern as ^{3}H-thymidine after administration of folate. There appears to be no correlation between the inhibition of FH_2 reductase which is seen in vitro and uptake of either of these isotopes. Although there was marked inhibition of enzyme activity following administration of methotrexate, there was no demonstrable effect upon DNA synthesis. Neither ^{14}C-thymidine nor ^{3}H-deoxyuridine was stimulated or depressed following administration of this agent. Enzyme inhibition was demonstrable for 7 days after drug treatment, however. Xanthopterin, which is known to stimulate DNA synthesis, had no effect upon enzyme activity but did stimulate DNA synthesis in the reported manner.

It appears from these results that the biochemical events which follow administration of these agents are complex and that the uptake of radioactive isotopes does not provide enough information to assess in vivo enzyme activity. It is hoped that this question will be answered by in vivo techniques now being employed.

C. 5-hydroxypicolinaldehyde thiosemicarbazone

Since last years report was written the work on this agent has slowed somewhat. However, reasonably convincing chromatographic evidence has been collected to show that the major metabolite in mouse urine is the glucuronide. Attempts to isolate this compound from the urine of two rabbits treated with 500mg each of the drug have so far been unsuccessful. However, it is still of considerable interest to isolate this metabolite and determine its structure because of the strong nauseating action of the drug in man.

An investigation was also conducted to see whether the drug showed any

variation in pharmacokinetics in different strains of mice. Three-commonly-used laboratory hybrid mice were examined together with their pure-bred parents. Blood-levels of free drug and metabolites, after a standard dose of the drug, were determined at intervals up to 2 hours after administration. The results showed remarkably similar blood decay curves for all strains examined and it was concluded that in these animals pharmacogenetic differences in the pharmacokinetics of 5-hydroxypicolinaldehyde thiosemicarbazone are of no significance.

D. Ellipticine and its Derivatives

Previous studies have shown tissue concentrations of ellipticine to be markedly dependent on route of administration. These results have been confirmed using radio-labeled 1-^{14}C-ellipticine, administered as a single dose of 250mg/kg. Concentrations of ellipticine in liver, spleen, kidney, heart, brain and blood were greatest after i.p. administration in solution, lowest after i.p. administration in suspension. Intermediate levels were noted after oral administration, but here too, administration of the drug in solution as the lactate was more effective than in suspension in Steroid Suspending Vehicle. The formation of metabolite(s) was evidenced by the presence in tissues, urine and feces of radioactivity that was not extractable into ethyl acetate. Non-extractable material accounted for 95% of the drug-derived radioactivity in urine. In tissues the major portion of the drug at all times over a 24-hour period as unchanged ellipticine, determined by spectrophotometric and paper chromatographic analyses. In liver, spleen, and kidney, 67, 74 and 77%, respectively, of ellipticine equivalents were present as the unchanged drug, regardless of the method of administration. In the feces, after i.p. administration of ellipticine in solution, approximately 50% of the amount of drug-derived radioactivity excreted over 6 days is accounted for as ellipticine; the remainder of the radioactivity was not extractable into ethyl acetate. Further excretion studies are currently in progress, and the nature of the metabolite(s) are under investigation.

E. Cyclocytidine (Anhydro cytosine arabinoside)

A major drawback to the usefulness of cytosine arabinoside as a chemotherapeutic agent is its rapid and extensive degradation *in vivo* to the inactive uracil arabinoside. Several attempts have been made to overcome this drawback including continuous intravenous infusion, combination with a pyrimidine nucleoside deaminase inhibitor such as tetrahydrouridine, conversion to a depot form such as cytosine arabinoside 5'-palmitate or even to implantation *in vivo* in a silastic container. However, none of these were dramatically different in their therapeutic action from ara-C although there were demonstrable changes in ara-C pharmacology and pharmacokinetics. Each of these approaches had its drawbacks, however, the demonstration by Japanese workers that cyclocytidine appeared to be resistant to deaminase action as well as being significantly more active against L1210 aroused considerable interest. An action group has been established to look into this compound's pharmacology. An L1210-resistant variant has been developed and will be used to investigate similarities and differences between the metabolism of cytosine arabinoside

and its congener. Radioactively-labeled drug has recently been obtained and will facilitate these studies greatly.

F. Dihydrofolate Reductase Radiometric Assay in vivo

As a direct outcome of studies on the disposition and metabolism of dihydrohomofolate it was found that its conversion in vivo to tetrahydrohomofolate could be inhibited by methotrexate. The degree of inhibition appeared to be proportional to the dose of methotrexate and it was reasoned that if ^{3}H-labeled dihydrofolate was used instead of dihydrohomofolate it should be possible to assess in vivo the degree of inhibition of dihydrofolate reductase by measuring the relative amounts of substrate and product present in the tissues at different times after administration of methotrexate. There would be several advantages to such a method over existing approaches, the major one being that it would be possible to accurately assess the inhibition in vivo at very early time periods after drug administration. Existing approaches require the removal of tissues and preparation of extracts and in the presence of free drug any unbound enzyme is rapidly incapacitated.

The capacity of liver and gut dihydrofolate reductase to convert ^{3}H-dihydrofolate to ^{3}H-tetrahydrofolate was readily demonstrable, and in the absence of methotrexate 95% or greater of the ^{3}H was present as tetrahydrofolate. Three hours after administration of graded doses of methotrexate there was a progressive, linear, dose-dependent increase in the relative amount of dihydrofolate present in the tissues accompanied by an equivalent decrease in the tetrahydrofolate levels. Single doses of methotrexate at therapeutic levels such as 1.5mg/kg did not inhibit the reduction of dihydrofolate to an appreciable extent but a dose of 15mg/kg inhibited the reaction about 85%. Recovery from the inhibitory effect of the single 15mg/kg dose of methotrexate was biphasic in both liver and gut and much more rapid than expected. The recovery pattern did, however, correlate very well with observed plasma decay curves in pharmacokinetic studies conducted in the Laboratory of Chemical Pharmacology. There are many ways in which the method can be employed; for example to compare the relative effects in vivo of new antifolates on dihydrofolate reductase activity. An earlier observation on the reversal of methotrexate toxicity and antileukemic effect by dihydrofolate can now be more readily explained since it is now known that inhibition of reductase activity is not nearly complete. This method, among other things, quite definitely sheds some doubt on the generally accepted mode of action of 4-amino antifolates since therapeutically active levels of the drugs apparently do not bring about the extensive and prolonged inhibition of dihydrofolate reductase that has generally been assumed to be the case.

G. Emetine and Other Antiparasitic Compounds

Earlier studies of the influence of schedule on the distribution of emetine in the tissues of normal mice have been expanded to include distribution patterns in leukemic animals, distribution following oral administration and drug levels in an increased number of tissues. Following administration of 16mg/kg on days 1, 5 and 9 emetine could be detected in the spleens, livers, kidneys,

stomachs, small intestines, lungs and hearts of leukemic animals. Peak drug levels are approximately the same in the livers, spleens and kidneys of both normal and leukemic animals. However, drug levels at the later time periods, that is, at 24 and 48 hrs after administration are significantly higher in the tissues of leukemic animals.

Emetine is inactive against leukemia L1210 when it is administered orally. Although the drug can be detected in the spleens of treated animals after administration of 40mg/kg orally, tissue levels are not as high as those seen following IP administration and the pattern of distribtuion is erratic.

In another study the antileukemic activity of diethylcarbamazine, a widely used antifilarial agent, was examined on several different schedules against leukemia L1210. It had previously shown no activity on a single dose trial in the primary screen. The drug showed no activity whatsoever on any schedule but as has been suggested by others, any agent which shows any kind of useful biological activity is worth examining more closely than usual in the primary and secondary evaluation systems. Other selected agents will be examined in the light of demonstrated biological activity in non-tumor screens especially in the antiparasitic area.

Significance to Biomedical Research and the Program of the Institute

The acquistition of knowledge pertaining to the physiological distribution and metabolic transformations of antitumor agents is a very important objective of the drug development program of Chemotherapy, NCI. This project is directed towards the goal of acquiring this information and also to undertake more fundamental studies to elucidate the relationship, if any, of physiological distribution to chemotherapeutic activity and/or toxicity. This program is an integral part of the scientific endeavor under the aegis of the Experimental Therapeutics Division of the Chemotherapy Program.

Proposed Course of Project

The project will continue to follow a course aimed towards the acquisition of relevant pharmacological, toxicological and biochemical data for input into the Linear Array for drug development. Concurrently, phenomena of a basic nature will be investigated inasmuch as they are related to the physiological effects, in animals, of experimental antitumor agents.

Honors and Awards:

Dr. J.A.R. Mead

"Relationship of Methotrexate to Folinic Acid in the Laboratory." Presented at Course on Protracted Cancer Chemotherapy by Ambulatory Arterial Infusion, Boston, Massachusetts, March 1972.

Publications:

Mishra, L. C. and Mead, J.A.R.: Further Evaluation of the Antitumor Activity of Homofolate and its Reduced Derivatives Against Methotrexate-Insensitive Tumors. Chemotherapy 17: 283-292, 1972.

Hardesty, C. T., Chen, D. and Mead, J.A.R.: The Use of Dihydrofolate Reductase as a Signal for Scheduling Optimal Treatment with Antileukemic Agents. Cancer Research 32: 458-461, 1972.

Tomchick, R., Weinstein, E. P. and Mead, J.A.R.: The Physiological Disposition of ^{14}C-Labeled 5-Hydroxypyridine-2-carboxaldehyde Thiosemicarbazone (NSC-107392) in Mice. Biochemical Medicine (in press).

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SUMMARY REPORT

LABORATORY OF CHEMICAL PHARMACOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 - June 30, 1972

The efforts in the Laboratory of Chemical Pharmacology continue to be primarily concerned with the elucidation of qualitative and quantitative aspects of the disposition (absorption, binding, distribution, metabolism, and excretion) and the mechanism of action of new and established antitumor agents as well as other model foreign compounds in man and laboratory animals. This requires in many instances the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. Of special concern in disposition studies is the establishment of rates and rate constants which govern the passage of drugs across membranes from one body compartment to another, the assessment of drug biotransformation or binding to cellular constituents or macromolecules, and the determination of the rate of elimination of the drug and its metabolites. When this information is considered together with a drug's chemical and physical properties as well as its route and schedule of administration, predictions can be made concerning the effective concentration of a drug or its metabolites in tumor and susceptible normal tissue - the receptor sites. These studies are particularly important since one of the major factors contributing to the lack of adequate drug response of the host is the failure of the drug to reach the target site in sufficient concentration to eradicate the tumor cells. The knowledge gained from such studies when considered together with biochemical, toxicologic, and cytokinetic data will hopefully permit the design of drug dose-levels and schedules by the clinician for more effective therapeutic use of antineoplastic agents in human malignancies.

During the past year a number of research projects were continued and several new studies were initiated. Some of the experimental findings are highlighted below.

The folate antagonist group of antitumor agents has been particularly appealing to this laboratory for comparative pharmacologic and biochemical studies because of the broad base of preclinical and clinical data that has been accumulated concerning these drugs within the Chemotherapy Program of the NCI and elsewhere over the past 15 years. This broad base of knowledge is now allowing us to make extensive pharmacokinetic analyses and certain empirical correlations to facilitate the transfer of laboratory animal data to man in an effort to improve prediction of therapeutic and toxic actions. Methotrexate (MTX) has been the primary model compound in these studies because of its importance as a clinically active antineoplastic agent.

In this laboratory, studies have continued on the development and refinement of a pharmacokinetic mathematic model for MTX to permit the "scaling up" of observed phenomena from one species to another. Of particular interest during the past year was refinement of the model to describe transport of methotrexate into bone marrow of rats. In the rat, bone marrow is the primary site of lethal toxicity by MTX. Transport was found to be a saturable process and the model successfully predicted behavior of the drug over a range of dose-levels. The model was also used to study the toxicity of MTX and reversal by citrovorum (CV) factor. The data suggested that prolonged low plasma drug concentrations ($< 1 \times 10^{-7}$M) resulting from enterohepatic circulation of the drug may be the primary cause of lethal intestinal toxicity in mice.

The enteric bacterial metabolism of MTX was further studied and in addition to 2,4-diamino-N^{10}-methylpteroic acid (APA), the 6-methyl,6-formyl, and 6-carboxy derivatives of 2,4-diaminopteridine were found to be metabolites of MTX in the urine and feces of rodents. APA was considerably less toxic than MTX in rodents in vivo and APA and the other metabolites identified were less effective than the parent compound as dihydrofolate reductase inhibitors. Efforts are being made to determine the quantitative significance of bacterial gut metabolism of MTX in man.

Another approach to correlating pharmacological data, particularly plasma levels of MTX, with effects on certain target organs (bone marrow, gastrointestinal mucosa, tumor) was initiated during the past year in collaboration with the NCI's Medicine Branch. This was done for MTX by studying its effects at certain time intervals and dose levels on the inhibition of DNA synthesis in bone marrow, gastrointestinal mucosa, and tumor cells of L1210 leukemia bearing mice and correlating these effects with concurrent plasma levels of the drug. The results indicate that this experimental approach may permit the prediction in man of the duration of inhibition of DNA synthesis in critical target sites by MTX from knowledge of plasma disappearance curves at various dose levels.

Studies have also continued concerning the effects of commonly employed corticosteroids, antibiotics and other antitumor drugs on the in vitro transport of MTX into L1210 cells. Vincristine and vinblastine enhanced the uptake of MTX into L1210 cells while the most significant inhibitors of transport were hydrocortisone, asparaginase, and cephalothin. An interesting correlation existed between these in vitro results and the antitumor activity of MTX in vivo. In brief, vincristine or vinblastine in combination with MTX markedly increased the survival of L1210 leukemia bearing mice as compared to MTX used alone. Conversely, hydrocortisone in combination with MTX significantly decreased survival. The results suggest that the concurrent use of cancer chemotherapeutic agents and other drugs may significantly alter cellular transport and ultimately antitumor activity of MTX.

The clinical pharmacology of MTX and other folate antagonists continues to be of considerable importance, with several areas still requiring study. For example, the importance of the biliary route of excretion for MTX and for 3',5'-chloromethotrexate (DCM) has not been determined as relates to

metabolic inactivation in normal and malignant human liver tissue. In a collaborative effort between the NCI and Uganda Cancer Institute, studies of biopsy specimens of normal and neoplastic liver of patients treated with DCM revealed that refractoriness of the tumor is not related to failure of the drug to penetrate the tumor tissue.

The enzyme aldehyde oxidase, which is present in mammalian liver, and plays an important role in the biological inactivation of certain folate antagonists, continues to be studied. An effort is being made to define the structural features necessary for a drug molecule to act as a substrate or inhibitor of the enzyme. In this regard the dimethyl and diethyl esters of MTX and DCM were synthesized and their binding affinities to the enzyme are being studied.

In extending studies to better understand the role of hepatic aldehyde oxidase in biological systems, the nitroreductase activity of this enzyme was examined using several potentially carcinogenic nitro-substituted N-heterocyclic compounds. It appears that the enzyme is capable of converting certain substrate compounds to the corresponding hydroxyamino metabolite (the "proximate carcinogen") under anaerobic conditions. The quantitative significance of this biological activation remains to be determined.

Investigations of the pharmacology of the unique group of antitumor agents, the nitrosoureas, continue to be an important area of study for this laboratory. During the past year, special emphasis was made in the study of the physiologic disposition of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) in patients during Phase I trial of the drug. One of the major objectives of this laboratory is to improve our ability to conduct clinical pharmacologic studies of antineoplastic agents during Phase I trials. Briefly, the results of ten patinet studies with the radioactive labeled drug indicate that MeCCNU is rapidly degraded after an oral dose with no intact parent drug detectable in any of the body fluids. The primary route of excretion is by the kidney, and interestingly, plasma of patients receiving the drug orally lacked L1210 murine leukemia killing activity in vitro. The pharmacologic disposition of the drug appears to be similar to other nitrosourea derivatives studied in this laboratory, particularly BCNU and CCNU.

The physiologic disposition of guanazole was studied in several laboratory animal species and preliminary studies have been conducted in patients during Phase I trial. The preclinical results indicate that the drug is rapidly excreted unaltered primarily by the kidney after a parenteral dose and is incompletely absorbed after an oral dose.

The toxicity and antitumor activity of gallium nitrate and several of its related salts were evaluated in rodents bearing tumors. Gallium nitrate demonstrated marked antitumor activity and is a potential candidate for clinical trial. In this regard, the distribution and excretion of Ga^{67} (used for scanning a variety of human malignancies) was studied in patients and the isotope showed prolonged tissue retention with elimination by both the urinary and fecal routes.

Plasma-protein binding studies of camptothecin were continued and 24 species including man have been examined. The degree of binding was correlated with the albumin and β-lipoprotein plasma levels. Of all mammalian species studied, protein binding was highest in man; this plasma binding may adversely affect therapeutic effectiveness of this alkaloid because of altered distribution and decreased plasma levels of "active" drug.

The comparative biliary excretion studies of antitumor drugs and other model compounds involving a variety of experimental animal species are of value in defining quantitative as well as qualitative differences in drug disposition. Comparative studies have also been extended to include drug metabolizing activity of microsomal and other subcellular fractions of mammalian livers. Included in these studies have been such compounds as camptothecin, procarbazine, MTX, cyclophosphamide, rifamycin SV, probenecid, and demethylchlortetracycline.

The availability of a large breeding and experimental monkey colony (600 animals representing 5 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, and biochemical studies in subhuman primates. Induction of hepatoma with N-nitrosodiethylamine (DENA) has provided a model for evaluation of potential antitumor drugs and for studies of various fundamental aspects of this tumor including α-fetoprotein production and its use as a marker. Also of particular interest is the induction of malignant lymphoma and lymphocytic leukemia in owl monkeys inoculated with Herpes saimiri virus. Preliminary results from chemotherapy trials suggest that cyclophosphamide and cytosine arabinoside are effective against these experimental malignancies in monkeys.

In addition to the basic laboratory research effort, each senior investigator shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with development of analytical-biochemical methodology, the study of pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and in in vitro predictive systems, and the development of pharmacokinetic methematical models. Approximately 12 agents are studied each year, each of which reflect the interest of the Chemotherapy Program area for developing antitumor drugs for the clinic. It is expected that the number of agents to be studied through the contract mechanism will increase with the expanding program on the national level.

Serial No. NCI-3502
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology and Drug Metabolism Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Folic Acid Antagonists

Previous Serial Number: Same

Principal Investigators: David G. Johns, Daniel S. Zaharko, Donald M. Valerino, Robert F. Zager, and Vincent T. Oliverio

Other Investigators: Robert L. Dedrick, Robert Lutz, and Vernon Wong

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS, and Clinical Branch, National Eye Institute

Man Years:

Total:
Professional:
Other:

Objectives:

I. Work on a Mathematical Model for Describing the Pharmacokinetics of Methotrexate (MTX) in Laboratory Animals and Man

Refinement and use of a pharmacokinetic model in order to gain insight into the limitations and further potential of folic acid antagonists. Development of more detailed in vivo kinetic models for individual organs such as bone marrow, intestinal tract, skin, eye and spleen.

II. Metabolism of Methotrexate by Intestinal Bacteria

Recent studies from this laboratory have shown the presence of at least two MTX metabolites in urine and feces of mice and rats after the administration of tritium-labeled MTX. The conversion appears to be carried out by intestinal bacteria of these species during enterohepatic circulation of the drug. The objectives of the present study are to identify these metabolites and to determine their toxicity and biological activity as inhibitors of dihydrofolate reductase, relative to that of the parent compound, MTX. Studies will then be carried out to determine the quantitative importance of this possible metabolic pathway for MTX in man. The long-term objective of these studies is to provide more complete data on the basic and clinical pharmacology of this important antineoplastic agent.

III. Transport of Methotrexate and Metabolites across Mammalian Cell Membranes

Studies are being conducted to determine the effect of commonly employed adrenal steroids, antibiotics, and other antitumor drugs on MTX transport into mammalian cells. Agents which inhibit and others which enhance MTX transport in the L1210 murine leukemia system have been found. Studies are now being conducted to determine the effects of these interactions on MTX antitumor activity in the L1210 system. Future work will include experiments to evaluate whether these interactions seen in the L1210 system can also be seen in human malignant cells.

IV. Clinical Pharmacology of Folate Antagonists

While many aspects of the clinical pharmacology of MTX and other folate antagonists have been elucidated, a number of gaps still exist in our knowledge of the pharmacology of these agents. Among areas which still require study are the relative importance of the biliary route of excretion for MTX and for 3',5'-dichloromethotrexate (DCM) and the rate of metabolic inactivation of DCM by normal human liver tissue and by primary hepatocellular carcinoma tissue. Advantage is being taken of an on-going project being conducted at the Uganda Cancer Institute by Dr. C. L. Vogel, Medicine Branch, NCI, on the clinical evaluation of DCM and MTX in primary hepatocellular carcinoma, to carry out studies on these areas of MTX and DCM pharmacology.

V. Metabolism of Folate Antagonists by Hepatic Aldehyde Oxidase

The enzyme aldehyde oxidase, present in the soluble supernatant fraction from mammalian liver homogenates, can catalyze the 7-hydroxylation of 3',5'-dichloromethotrexate (DCM). The non-halogenated parent compound of this series, MTX, is subject to 7-hydroxylation by aldehyde oxidase from rabbit liver, but not from most other mammalian species. Since the oxidation results in biological inactivation of these drugs, further knowledge of the reaction is of direct relevance to the pharmacology of MTX, DCM, and other folate antagonists. Structure-activity studies are being carried out to define the features required for optimal binding to the enzyme, in order to permit the design of effective inhibitors of this inactivation reaction *in vivo*.

Methods Employed:

Chromatographically pure MTX-3',5'-T was employed throughout. Drugs were administered by the intravenous, intraperitoneal, or intraduodenal routes. Standard surgical procedures and specimen collection techniques were employed throughout. In other experiments, mouse caecal contents were incubated with tritium-labeled MTX and the metabolites recovered. MTX metabolites were characterized by their chromatographic behavior in several

systems and by their ultraviolet and infra-red absorption spectra. Large amounts of MTX metabolites were prepared by cleavage of MTX by bacterial carboxypeptidase.

Tentative identifications were confirmed by synthesis, followed by comparison of the properties of the synthetic compounds with those of the naturally occurring metabolites. The toxicity of the metabolites relative to that of the parent MTX was estimated. The activity of the metabolites as inhibitors of dihydrofolate reductase from mouse L1210 leukemia cells was determined relative to that of MTX. In patients with primary hepatomas receiving antifolate therapy at the Uganda Cancer Institute, frozen samples of plasma, urine, liver biopsy, and other biological materials are shipped to the Laboratory of Chemical Pharmacology, NCI for determination of drug levels and of DCM-oxidase enzyme activity by a direct spectrophotometric method. The methodology employed for short-term in vitro uptake and efflux studies of MTX in L1210 leukemic cells and human leukemic blast cells was adapted from the procedures of Goldman et al. (J. Biol. Chem. 243:5007, 1968). Survival studies were done using the transplantable, ascitic L1210 tumor in CSF_1 mice. In addition, ascites cells from these mice were used for in vitro MTX uptake studies and for experiments to determine the number of ascitic tumor cells per mouse.

Major Findings:

I. Model Applications

a) Extensive kinetic studies have shown that MTX transport into the bone marrow of rats is a saturable process. This process can be described by a mathematical equation of the Michaelis Menten type. This process was coupled with blood flow, extracellular and intracellular spaces, and strong saturable binding by the enzyme dihydrofolate reductase and then included into the overall pharmacokinetic model. In this form predictions of bone marrow drug concentrations resulting from doses ranging from 0.05 mg/kg to 25 mg/kg were successful. These total marrow drug concentrations could be categorized into drug in: tissue blood, tissue extracellular fluid, tissue intracellular fluid (enzyme bound and free).

Similar type analyses are anticipated for the data collected in these experiments for tissues such as gastrointestinal tract, skin and spleen.

b) Application of the model to further the understanding of the toxicity of MTX and its reversal by citrovorum factor has been attempted. Data suggest that the prolonged concentration of the drug at very low plasma concentrations ($<1 \times 10^{-7}$M) is the primary cause of intestinal toxicity and resultant death of mice. Reversal of this toxicity by delayed citrovorum factor (as long as 24-48 hours) or by in vivo injection of carboxypeptidase-G (Dr. Bruce Chabner's finding) which detoxifies plasma MTX gives further support to this kinetically identified hypothesis.

Application of the pharmacokinetic model in conjunction with a cell kinetic model (developed by Dr. Ken Bischoff) to attempt predictions of L1210 cell kill in mice has had a preliminary trial with some success for the drug Ara-C.

II. Enteric Bacterial Metabolism

Studies have continued on the isolation and identification of metabolites of methotrexate in rats and mice particularly those rapidly migrating (pre-MTX and pre-APA) metabolites on DEAE-cellulose. Multiple chromatographic techniques were used to purify several unconjugated aminopteridines present in the urine and feces of these animals. These techniques involved column chromatography on DEAE-cellulose, Seph dex G-25 and Phosphocellulose followed by paper chromatography. Those 2,4-diaminopteridines that were identified in the feces bore either a methyl, formyl or carboxyl group at carbon #6 in the pyrazine portion of the pteridine nucleus. At least one 2,4-diaminopteridine has been isolated from the urine of rats. This pteridine exhibited identical chromatographic and spectroscopic behavior with 2,4-diamino-6-methylpteridine.

All of these unconjugated 2,4-diaminopteridines are less effective inhibitors than MTX of dihydrofolate reductase obtained from L1210 cells. Zakrzewski (Journal of Biological Chemistry 238: 4002, 1963) reported that the K_i at pH 6.0 for 2,4-diamino-6-methylpteridine was 4.7×10^{-7}M. The K_i for 2,4-diamino-6-formylpteridine at pH 6.0 was 8.1×10^{-6}M. Our data indicate that the K_i for 2,4-diamino-6-carboxylpteridine was 9.1×10^{-6}M.

Toxicological studies were also conducted on the major fecal metabolite 4-amino-4-deoxy-N^{10} methyl pteroic acid (APA). The LD_{50} for APA in CDF_1 mice, injected i.p. daily on 2 consecutive days was found to be 260 mg/kg while that found for MTX was 58 mg/kg. APA is also a less effective inhibitor than MTX of L1210 cells in vitro.

Other studies were conducted on possible mechanisms for the cleavage of methotrexate at the C_9-N_{10} bond. It was found that hydrogen peroxide (produced a number of oxidative enzymes) and Fentons Reagent (a model for biological oxidations) will cleave MTX at the C_9-N_{10} bond. It is possible that free radicals are responsible for this mode of degradation.

Preliminary studies have revealed a possible interaction between cholestyramine and methotrexate. An increase in the oral toxicity of MTX was observed when cholestyramine (5 gms/kg) is administered 2 hours and 15 minutes prior to and 2 hours and 6 hours after the oral administration of MTX.

III. Transport Studies

The in vitro uptake of MTX by sensitive L1210 cells grown in spinner culture is unidirectional until high affinity intracellular binding sites (presumably DHFR) are saturated. The rate of uptake and total intracellular concentration of MTX at a given point on the uptake curve are both proportional to the extracellular MTX concentration. Cells preloaded with MTX when suspended in MTX-free buffer, rapidly efflux MTX to a concentration of 6-9 mμM MTX/gm dry cell weight - the approximate level of intracellular DHFR.

Hydrocortisone, methylprednisolone, L-asparaginase and cephalothin (Keflin) were found to have an inhibitory effect on the in vitro uptake of MTX by L1210 cells. The vinca alkaloids (vincristine and vinblastine) enhanced MTX uptake by L1210 cells. These effects were dose related and the inhibition appears to be of a competitive nature. Pre-incubation of L1210 cells with vinca alkaloids results in enhancement of MTX uptake in subsequent in vitro uptakes. No effect on MTX efflux has been observed for any compound examined.

In vivo experiments have shown that inhibitors of MTX transport (hydrocortisone and L-asparaginase) can antagonize the MTX induced prolongation of survival in L1210 mice. Also, vinca alkaloids can act synergistically with MTX to prolong the survival of L1210 mice and even result in a percentage of "cures".

IV. Clinical Pharmacology

In biopsy specimens of normal and neoplastic liver obtained from three patients with hepatocellular carcinoma after the administration of DCM, no significant differences have been noted in the levels of the drug present in the tumor and in the surrounding normal tissue. The refractoriness of this tumor to therapy with DCM does not appear to be due to failure of the drug to penetrate the tumor tissue.

In ancillary studies, the rate of MTX secretion in human milk was determined. After oral administration of MTX at a dose-level of 15 mg/m^2 to an East African nursing mother with choriocarcinoma, the drug was found to be excreted in milk at low but readily detectable concentrations. The highest milk:plasma concentration ratio, 0.08:1, was observed 10 hr after administration of the drug. Since information on the lipid solubility of MTX, necessary for the interpretation of these data, was not available, n-butanol:water partition coefficients were determined over a wide range of pH. The kinetics of the MTX secretion process correspond to those which would be anticipated for a weak organic acid transferred from plasma to milk by passive diffusion of the non-ionized form.

V. Metabolism of Folate Antagonists by Hepatic Aldehyde Oxidase

In efforts to delineate the structural features necessary for tight binding of substrates and inhibitors of hepatic aldehyde oxidase, the dimethyl and diethyl esters of MTX and DCM have been synthesized and their substrate activities determined. The MTX esters are rapidly converted to the corresponding 7-hydroxy compounds by rabbit liver aldehyde oxidase, with apparent affinities for the enzyme more than 10-fold greater than that of the non-esterified parent compound (apparent K_m-values for the esters, 0.01 mM, vs. 0.18 mM for MTX). For the rat liver enzyme, MTX lacks detectable substrate activity at pH 7.5, while both MTX esters undergo rapid oxidation. The 3',5'-dichloroMTX esters also show enhanced substrate activity for both enzymes, but quantitative studies with the latter compounds were hampered by their poor water solubility. The enhanced activity seen with the lipid-soluble esterified substrates is compatible with the presence of a hydrophobic region in close juxtaposition to the substrate-binding site of the enzyme.

Significance to Bio-medical Research and the Program of the Institute:

Among the active antitumor folic acid antagonists, MTX is the most widely used, and is clinically effective in the treatment of acute lymphocytic leukemia, choriocarcinoma, Burkitt's lymphoma, primary hepatocellular carcinoma, and epidermoid cancer of the head and neck. Because of the great clinical importance of MTX, a complete knowledge of its pharmacology is of practical value. With this established agent it is envisioned that additional information on its distribution and metabolism in a variety of species, and a further understanding of its uptake characteristics into malignant cells alone or in the presence of other drugs, will lead to its improved use and the design and use of newer more effective drugs.

Proposed Course:

1. Continue studies to quantitate in vivo transport in additional normal tissues and also in a variety of tumors. The long-term goal is to utilize this kinetic information to schedule optimal therapeutic doses and schedules in experimental animals to illustrate the utility of maximal use of pharmacokinetic information in conjunction with biochemical and cell kinetic knowledge.

2. To initiate studies concerning the effect of drug combinations, drug interactions and prior drug biological effect on the kinetic parameters established for normal and neoplastic tissues.

3. Examination of human urine and feces after the administration of tritium-labeled MTX for the presence of MTX metabolites utilizing the same methods as those described above for MTX metabolites in the mouse.

4. If MTX metabolites are found in man, the effect of antibiotic administration on the levels of such metabolites and on the toxicity of MTX in man will be determined.

5. To determine what differences in MTX transport (if any) divide resistant from sensitive human leukemic cells.

6. To continue study of drug-drug interaction of MTX in L1210 cell system and to extend similar investigations to in vitro study of human leukemic cells and ultimately to in vivo studies in animals and humans. To extend this type of research to examine other chemotherapeutic agents in appropriate animal tumor systems.

7. Over the longer term, the course of clinical pharmacologic studies will be determined by the clinical results obtained in the treatment of primary hepatomas with DCM. It is hoped that sufficient data will be accumulated to test the hypothesis of a possible selective advantage of DCM over MTX in the treatment of these tumors. Ancillary clinical pharmacological studies on biliary and mammary gland secretion of DCM and MTX will also be carried out as clinical material becomes available; these studies are of value since some of this data is not presently available, but will be required for the complete development of a pharmacokinetic model of MTX and DCM distribution in man.

8. To define further the structural requirements for substrate activity of the DCM-oxidizing enzyme aldehyde oxidase, in order to permit the design of agents resistant to inactivation by the enzyme, and also to aid in the design of effective inhibitors of the enzyme in vivo.

Publications:

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Serial No. NCI-3505
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Potential Carcinogenic Activity and Chronic Toxicity of Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

The susceptibility of newborn and adult animals to chemical carcinogens and, particularly, drugs of interest in cancer chemotherapy and antiviral agents and those to which the human population may be exposed is of vital necessity and of particular relevance to the program of NCI. The characterizations and treatment of such tumors is of both theoretical and practical importance. The specific objectives are:

To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy, such as alkylating agents, antimetabolites, agents that intercalate with DNA, polymers and various antiviral agents.

To characterize and transplant carcinogen-induced tumors as new tools for experimental cancer chemotherapy.

To compare the susceptibility of newborn vs. adult animals to chemical carcinogens.

Methods Employed:

Usual biologic, pharmacologic, and histopathologic techniques.

Major Findings:

Tilorone and guanazole were given both in single and multiple injections to mice and the animals were held for various periods of time then sacrificed and all tissues were examined grossly and also microscopically for evidence of tumor and other pathology. Neither of these agents appear to be highly carcinogenic in CDF_1 mice.

Procarbazine is being further evaluated for carcinogenic activity both alone and in combination with other antitumor agents since combination chemotherapy including procarbazine is being utilized extensively in the clinic for treatment of Hodgkin's. Procarbazine is highly carcinogenic when administered to mice, rats and also has induced leukemia in monkeys in our colony.

Various tRNA's were evaluated for carcinogenic activity in two strains of mice, thus far, these tRNA's have not been found to be carcinogenic.

Various synthetic RNA's which stimulated interferon production or the immune system are undergoing evaluation for potential carcinogenic activity. Poly I·Poly C did not exhibit significant carcinogenic activity when evaluated on a variety of doses and schedules in General Purpose mice.

Gallium nitrate and periodically related metal salts were evaluated for acute and chronic toxic effects in mice and rats. The major findings for each Group IIIa salt are summarized: Aluminum nitrate caused marked congestion of the liver and lung in mice and an intense chemical peritonitis with ascites formation and marked congestion of the lung in the rat. Gallium nitrate treatment resulted in pneumonitis and chronic inflammatory infiltration of the alveolar septa and capillaries and some renal tubular damage in mice and slight-to-moderate chemical peritonitis, intersitial pneumonitis with slight pleural effusion, and moderate liver and kidney damage in rats. Indium nitrate treatment of mice caused myelopoiesis in the liver, moderate interstitial pneumonitis, spermatogenic arrest, moderate bone-marrow hyperplasia, and congestion or hemorrhage in the peritoneum and/or pleural cavity. In the rat, Indium nitrate caused interstitial pneumonitis and moderate inflammation of hepatic serosa and parenchyma. The toxic effects of Thallium chloride occurred primarily in the lungs of both mice and rats. Both species had marked alopecia beginning about 20 days after the first dose of $TlCl_3$. Whether pneumonitis seen with all 4 salts was directly related to drug treatment could not be established.

Significance to Bio-medical Research and the Program of the Institute:

Several drugs used as anticancer agents are known to be carcinogens. Few, however, have exhibited such strong carcinogenic activity as procarbazine, and none elicit such a wide spectrum of tumors in mice and rats. With the clinical use of procarbazine, it is essential to carefully delineate its carcinogenic potential, not only in rodents, but also in primates.

Guanazole is chemically related to the triazole that is used on cranberries which produced thyroid tumors in rodents. The possibility that guanazole may have a similar action was investigated. The possible carcinogenic activity of other antitumor agents is not only of clinical importance, but theoretically, they may be of importance to help understand the malignant process.

The acute and chronic toxicity of anticancer agents is of vital importance and except for chronic testing of antineoplastic agents, no further work is projected in this area, but is being left to the Laboratory of Toxicology.

Proposed Course:

New antiviral and anticancer agents of clinical interest will be evaluated as potential carcinogens. Tumors that occur from various carcinogens will be evaluated for their applicability to programs for antitumor testing.

Publications:

None

This report was previously entitled "Chemical Carcinogenesis".

Serial No. NCI-3506
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Pharmacology of Alkylating Agents and Other Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Susan M. Fabro

Other Investigators: James Arseneau, George Canellos, I. Zarembok, S. Larson, R. Aamodt and G. Johnson

Cooperating Units: Medicine Branch, Department of Nuclear Medicine, NIH and Baltimore Cancer Research Center, Baltimore, Md. 21211

Man Years:

Total:	3.0
Professional:	1.5
Other:	1.5

Project Description:

Objectives:

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

The possible embryotoxic effects of anticancer agents and antiviral agents that may be used clinically or more generally in non-malignant conditions are being investigated.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, immunologic, and histopathologic techniques.

Major Findings:

Antitumor Activity of Gallium

The toxicity and antitumor activity of gallium nitrate and periodically related salts of aluminum, indium, and thallium were examined. The order of increasing lethality in mice and rats was $Al(NO_3)_3<Ga(NO_3)_3<TlCl_3<In(NO_3)_3$. All 4 metal salts were active in prolonging the lifespan of rats inoculated with ascites Walker carcinosarcoma 256, but non of the metal salts were active in mice bearing ascites leukemias, plasma-cell tumors, or Ehrlich carcinoma. Gallium nitrate was also active in inhibiting the growth of 6 of 8 solid tumors in rodents. Al^{+3} and In^{+3} had less activity, and thallium chloride was inactive. Gallium nitrate, therefore, has potential usefulness for treatment of various solid tumors in humans.

Antitumor Activity of Tilorone Hydrochloride

Tilorone hydrochloride, a synthetic small molecular-weight inducer of interferon, had antitumor activity against Walker carcinosarcoma 256 and the reticulum cell sarcoma A-RCS in rodents. Tilorone hydrochloride was inactive when tested in several other rodent tumors including leukemias L1210 and P388. Tilorone hydrochloride also had cytotoxic activity in vitro against Walker carcinosarcoma 256, leukemia L5178Y, and the Novikoff hepatoma. Although tilorone did not prolong the survival time of mice with leukemia L1210 or P388, it or similar oral inducers of interferon, may have therapeutic value in man in maintaining remission in acute lymphocytic leukemia and/or as therapy in leukemic patients for life-threatening systemic viral infections caused by DNA or RNA viruses. Moreover, the fact that a synthetic small molecular weight interferon inducer has antitumor activity lends additional support to interferon induction as a mechanism for the antitumor action of compounds such as poly I·poly C.

Antitumor Activity of Rifamycin SV

The antibacterial, antiviral drug rifamycin SV was evaluated for antitumor activity in vitro and in vivo. Rifamycin SV was cytotoxic to three tumor lines in tissue culture and also had antitumor activity in vivo against the ascitic Walker 256 carcinosarcoma.

Rifamycin SV had no antitumor activity in vivo against leukemia L1210 and P388 using the injection schedule of once/day. A comparison of the antitumor activity of three antiviral agents: rifamycin SV, tilorone, and poly I·poly C, against Walker 256 carcinosarcoma showed rifamycin SV and poly I·poly C to be comparable in antitumor activity against this tumor (100-125% increase in MST) but tilorone had greater effectiveness than either rifamycin SV or poly I·poly C giving an increase in median survival of 700% over controls. The fact that these three antiviral agents have antitumor activity against the ascitic Walker 256 carcinosarcoma suggests that this may be a useful tumor model for evaluating antiviral drugs for antitumor activity.

Antitumor Activity of Hycanthone

Hycanthone, a thioxanthone derivative, has been shown to be highly active in the treatment of schistosomiasis both in animals and in man. About 100,000 people have been treated with this drug in South America and in Africa, and the apparent efficacy and safety of hycanthone have generated widespread enthusiam and larger trials. Hycanthone was evaluated for experimental antileukemic activity for the following reasons: (1) its high degree of activity against schistosomiasis, with none of the haematological toxicity often seen with other antileukemic agents; (2) it has been shown to be mutagenic in bacteria; (3) and, most important, it contains certain structural features which are present in other antileukemic drugs (for example, a planar aromatic nucleus, the presence of a keto group in this nucleus, and a diethylamino moiety in the side-chain).

Hycanthone inhibited 50% growth of L1210 cells *in vitro* at doses of 5 μg/ml and had *in vivo* activity against leukemia L1210 and leukemia P388 giving increases of median life span of 90 and 120% respectively. The cytotoxic effect of hycanthone against L1210 *in vitro* and its antileukemic activity *in vivo* as well as its low toxicity in patients being treated for schistosomiasis strongly suggests that this compound should be evaluated for antileukemic activity in man.

Disposition of ^{14}C-Guanazole

^{14}C-guanazole distribution and excretion was studied in several species. Distribution studies in mice one half hour after i.p. administration show high specific activity in the small and large intestines with contents, the kidney, bladder, and spleen - the specific activity in these tissues remains elevated at 1 hour, 1 1/2 hours and 2 hours. In contrast tissue specific activity of brain was significantly less than any other tissue at 1/2 hours, 1 hour and 1 1/2 hours after guanazole administration.

Excretion studies in mice after i.p. or s.c. administration showed that 50% of ^{14}C was excreted in the urine within the first hour and by the end of two hours approximately 88% of the ^{14}C was in the urine with an additional 2% being excreted by the end of the third hour. At all time periods only 1% or less was excreted in the feces. Excretion of ^{14}C four hours after oral administration of guanazole showed higher amounts in the feces and only 70% of ^{14}C in the urine suggesting that higher doses are necessary for antitumor activity by oral administration. Excretion of ^{14}C-guanazole was also rapid following parenteral administration to rats and dogs with over 80% being excreted in the urine within six hours. In addition to the animal experiments, studies with tracer doses of guanazole or doses of 5 grams M^2 in a 24 hour infusion were performed in patients with malignant disease. When tracer doses of ^{14}C-guanazole were given 50% of the ^{14}C was found in the urine within six hours. Approximately 60% of radioactivity was found in the urine at the end of a twenty-four hour infusion and 80 hours after infusion started 91% could be recovered.

Chromatographic studies with animal and patient urine indicate that the majority, if not all, the radioactivity found was unchanged parent compound.

Disposition Studies with ^{67}-Gallium

Studies of Ga^{67} distribution and excretion in patients revealed a two component whole body retention curve with a shorter retention in plasma (T 1/2 = 0.7 days) and a prolonged retention in tissue (T 1/2 = 13.8 days). Excretion of Ga^{67} occurred by both urinary and fecal routes.

Ga^{67} was used to scan patients with a variety of malignancies. Tumor uptake was demonstrated in Hodgkin's disease, lymphosarcoma, malignant melanoma and also in tissue myeloblastomas in one patient in whom concentrations in circulating lymphocytes was also demonstrated.

Based on the above observations and the previously mentioned antitumor activity of gallium as well as scanning data from other laboratories, a phase I protocol has been submitted for a clinical trial of gallium in human malignancy.

Significance to Bio-medical Research and the Program of the Institute:

Careful evaluation of the effect of potential and known antitumor compounds in induced tumor systems should be made until sufficient data is available to judge their usefulness in screening compounds for ultimate clinical trial. The possible embryotoxic and/or teratogenic effects of the various anticancer and antiviral drugs is of clinical importance and also may help shed light on nutritional requirements of fetus and on possible mechanisms of contraception. A rational approach to the design, selection and testing of new anticancer agents is of utmost importance and supplements the screening approach of Drug Research and Development Branch. Knowledge of the metabolic fate of various anticancer agents, their plasma t 1/2, their route of disposition, and their interaction with other drugs will lead to better therapeutic treatment in man and will aid in the design of agents for experimental animal trials.

Proposed Course:

1. Use of induced tumors (primary and transplanted) for chemotherapy will be continued.

2. Studies of the metabolic fate of ^{14}C-guanazole in man and of newer anticancer agents in animals and man will continue.

3. Studies on the mechanism of the antitumor action of tilorone will continue and the antitumor activity of various interferon inducers and interferon *per se* will be evaluated in various models.

4. Studies of the embryotoxic effects of newer anticancer agents will continue.

5. A rational approach to the design, selection, and testing of potential anticancer agents will continue.

6. The role of the lymphatic system in drug disposition in general and cancer chemotherapeutic agents in particular will be investigated.

Publications:

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3. Hart, M. M., and Adamson, R. H.: Antitumor Activity and Toxicity of Salts of Inorganic Group IIIa Metals: Aluminum, Gallium, Indium, and Thallium. Proc. Nat. Acad. Sci. 68: 1623-1626, 1971.

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Serial No. NCI-3509
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Carcinogenesis, Chemotherapy and Biological Markers in Primates

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Pelayo Correa

Other Investigators: Jacqueline Whang-Peng, Robert Gallo, T. Phillip Waalkes, Dan Dalgard, Hugh Bonner, Robert McIntire, and Dharam V. Ablashi

Cooperating Units: Laboratory of Pathology, Viral Leukemia and Lymphoma Branch, Human Tumor Cell Biology Branch, and Medicine Branch, NCI; Hazleton Laboratories, Inc., Falls Church, Va., and Bionetics Research Laboratories, Kensington, Md.

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic in man. In addition, the induced primate tumors will hopefully be useful as model tumor systems for man and for development of biological markers. Specifically the objectives are:

To study the effect of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

To evaluate the long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To evaluate known and suspected oncogenic virus in subhuman primates and to utilize these tumors for chemotherapy with antiviral and antitumor agents.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

Methods Employed:

Usual pharmacologic, biochemical, clinical, and anatomical pathologic techniques and cytological and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years. In addition, we have obtained owl monkeys (Aotus trivirgatus) and are attempting to establish a breeding colony of this species.

Major Findings:

General Information:

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed in the breeding colony and information concerning the methods utilized in rearing of the newborns. These procedures have not been signficantly altered and are, therefore, not included in this report.

The present colony consisting of 600 animals is comprised of five species: Macaca mulatta, Macaca irus, Cercopithecus aethiops, Galago crassicaudatus panganiensis, and Aotus trivirgatus. Of these, 450 are experimental; the remaining 150 are adult breeders which supply the newborns for experimental study.

All animals are housed in an isolated facility which contains only animals committed to this study. Most animals with the exception of the breeding colony are housed in individual cages. As newborns are produced by the breeding colony, they are delivered to the nursery area which is staffed on a 24-hour basis. Compound administration is generally initiated immediately.

Various physical and chemical parameters are monitored in each animal to evaluate the general health status and any evidence of tumorigenesis. Surgical biopsies of tumor specimens are obtained when indicated and submitted to the Laboratory of Pathology for histopathologic evaluation. All animals which die or are sacrificed are carefully necropsied and tissues examined microscopically.

No major disease problems have been encountered during this period.

Compounds of Particular Interest:

N-Nitrosodiethylamine (DENA)

This compound has continued to demonstrate potent carcinogenic properties. In general administration by the intraperitoneal route continues to produce a very high incidence (approximately 100%) of liver tumors which metastasize readily to the lungs and lymphatic tissues. The induction time is inversely affected by the level of administration and the frequency of administration, but generally ranges from 13 to 20 months. The shortest induction time has been eight months.

To date, liver tumors have been induced in 80 animals utilizing DENA. Fifty of these have resulted following intraperitoneal injections generally given every 14 days at a maximum level of 40 mg/kg. The oral administration of DENA at the same level, but given five times weekly resulted in a lower incidence and longer induction time. A total of 30 hepatic cell carcinomas were induced by this method.

Within the last two years we have started a group of rhesus and cynamolgus monkeys on i.p. DENA after the newborn monkey has been serum alpha-fetoprotein (AFP) negative for two consecutive weeks (generally by age of 1 month). By employing gradually increasing doses (10, 20 then 40 mg/kg) so that the monkey is receiving 40 mg/kg i.p. every two weeks by three months of age, we believe that we can induce tumors with an incidence of 100%. The average latent period for development of tumors kept on this schedule (average of 11 monkeys thus far with tumor) was 12 months. Evidence of tumor has been preceeded in all these animals by the presence of AFP generally 6 months before any evidence of tumor. Six monkeys were sacrificed when AFP positive and upon gross examination of cut sections and microscopic examinations of 100-300 sections no evidence of tumor was found. In all other monkeys in this group with a positive AFP no tumor was found during a laparotomy and biopsy of the liver. Several of these monkeys have subsequently developed tumor even though DENA administration was discontinued following laparotomy. Therefore AFP is a predictive test of monkeys that will eventually have tumor and also indicates a precancerous change which may or may not be susceptible to immunotherapy or prophylactic chemotherapy.

Chemotherapeutic control of tumors has been attempted. Methotrexate, camptothecin, poly I·poly C, and L-asparaginase and gallium therapy have been given to a small number of animals. Total regressions were not affected, but some evidence of growth suppression by both gross measurements, AFP levels and histopathology was seen.

1-Nitrosopiperidine (PIP)

Five out of ten monkeys receiving PIP orally at a dose of 400 mg/kg five times a week have developed liver tumors after periods of 36 to 89 months on compound. Four of the five monkeys were tested for AFP when diagnosed for tumor following biopsy and two had a positive serum AFP.

N-Isopropyl-α-(2-methylhydrazino)-p-toluamide (Procarbazine)

Procarbazine given subcutaneously and orally has induced myelogenous leukemia in two monkeys which received the compound for 17 months and 5 1/2 years respectively. In addition, two monkeys have died with hyperplastic bone marrow, three with ulceration of the esophagus and three with hyperplasia of the urogenital tract.

Attempts to transmit these leukemias by intracerebral inoculation in newborn Rhesus monkeys (M. mulatta) have so far failed, although this method has been successful for transplanting monkey hepatoma after growth in tissue culture. No viruses have been detected in either of the leukemias by electron microscopic examination of tissue, by animal inoculation or tissue culture, although studies are not yet completed on the second leukemia.

Other Carcinogens

Two polycyclic hydrocarbons have been used in higher primates: 3-methylcholanthrene and 3,4,9,10-dibenzpyrene. These carcinogens have been administered by oral and single and repeated intradermal and subcutaneous injection. Thirty-six monkeys are still alive after five years of treatment and up to an additional observation period of five years. Thus far, no sarcomas or carcinomas have been observed in these higher primates but tumors have been induced in lower primates e.g., tree shrews and galagos 12 months after a single injection of a variety of polycyclic hydrocarbons. Therefore, it seems possible that the prosimians which include tree shrews, lemurs, lorises, and tarsiers may be more closely related to the rodent than to the higher primates in their reactions to polycyclic hydrocarbon carcinogens.

Two fluorenylacetamides have been given by the oral route (2-FAA and 2,7-FAA) and the copper chelate of N-hydroxy-2-fluorenyl-acetamide has been injected subcutaneously. The copper chelate has produced foreign body granulomas and cysts at the site of injection and the orally administered fluorenylacetamides have produced fatty degeneration of the liver but no tumors have been induced with these compounds in our colony.

The two azo dyes, DAB and 3'-methyl DAB have been administered orally and have produced toxic hepatitis, fatty degeneration of the liver, ileocolitis, gastritis, and pulmonary thrombosis and periarteritis but no tumors have developed even though monkeys have received these compounds in their diet for five years.

Twenty-four monkeys are still alive after receiving ethyl carbamate orally at doses of 250 mg/kg, five times a week for a period of five years. Thirteen monkeys were also given 50 r whole body radiation for ten weeks (total of 500 r) as well as receiving ethyl carbamate orally. Thus far no animals receiving ethyl carbamate alone or in combination with radiation have developed leukemia or other tumors.

Methylnitrosourea administered orally at doses of 20 mg/kg five times a week or 5 mg/kg i.v. weekly has thus far not induced any tumors.

Aflatoxin B_1 given orally at 0.8 mg/kg 3 times/week or i.p. at doses of 0.25 mg/kg weekly has thus far not induced any tumors in monkeys. Animals biopsied or dying have shown toxic hepatitis with bile plugs in small canaliculi and proliferation of pseudotubules or small bile ducts, severe liver necrosis, abdominal distension, pulmonary edema, congestion and emphysema but no hepatomas or other tumors.

Monkeys receiving the artificial sweetners cyclamate or saccharin have thus far not developed cancer. Monkeys receiving DDT orally at 20 mg/kg five times weekly have not developed any clinical toxicity or cancer after 2 years of treatment.

N-methyl-N'-nitro-N-nitrosoguanidine has recently been given to newborn monkeys orally with the hopes that this compound will induce carcinoma of the stomach and duodenum as it does in rats.

Herpesvirus saimiri (HVS)

Fifteen of twenty owl monkeys inoculated with HVS propagated in owl and African green monkey kidney cell cultures developed malignant lymphoma with lymphocytic leukemia. The virus was recovered from these monkeys and identified as HVS. The disease had a course of 55 to 80 days and was characterized by generalized lymphadenopathy and lymphocytic leukemia with circulating actively motile lymphocytes. The lesions were characterized by the obliteration of normal lymphoid tissue architecture by neoplastic lymphocytes and by neoplastic lymphocytic invasion of almost all organs. No virus was detected in the tissues by electron microscopy, however, upon co-cultivation HVS was recovered.

Chemotherapy of owl monkeys with malignant lymphoma and lymphocytic leukemia has been started and preliminary results suggest that both cytoxan and cytosine arabinoside are effective in the treatment of owl monkeys with this disease. In addition several agents were examined for their effects on the multiplication of HVS *in vitro*. The multiplication of HVS *in vitro* was

inhibited by cytosine arabinoside, adenine arabinoside, and tilorone, but not by rifamycin SV.

Biochemical Studies of Tumors and Diagnostic Studies of Monkeys with Tumors

Hepatomas induced in monkeys by the administration of DENA were used in tRNA studies. AFP not normally found in adult monkeys was present in the serum of the majority of the animals used with the liver tumor. With tRNA from Escherichia coli $K_{12}W_6$ or from normal adult monkey liver or hepatoma as methyl acceptor, an increasing degree of methylation was found with the tRNA methylases in tissue extracts from normal adult monkey liver, premalignant liver, liver from newborn monkeys, and hepatoma, respectively. These observations suggest that a change in specificity of tRNA methylation occurs during chemical carcinogenesis with N-nitrosodiethylamine. The increase in tRNA methylation by extracts of premalignant liver also suggests that changes in tRNA methylase activity occur prior to, or as an early manifestation of, malignant conversion.

Further study showed the presence of an inhibitor of tRNA methylases in extracts from normal adult monkey liver but not in extracts of normal newborn liver and hepatoma. Thus, multiple factors may be involved which account for the differences observed in the tRNA methylases.

Preliminary studies using ^{67} Gallium administered i.v. or ethiodol administered intrasplenically indicated that these agents concentrate in the liver and in hepatic tumors, the level being higher in tumor tissue and therefore these agents may well be useful in the diagnosis of liver tumor and in the evaluation of effects of chemotherapeutic agents on liver tumors.

Significance to Bio-medical Research and the Program of the Institute:

The susceptibility of non-human primates to known rodent carcinogens and to possible carcinogens in man is of theoretical, practical, and economic importance. In addition, studies of the carcinogenic potential of antitumor agents will indicate whether these compounds should or should not be used clinically in patients without neoplastic disease for organ transplantation and in treating arthritis and other diffuse collagen disorders. The susceptibility of the newborn monkey vs. the adult monkey to carcinogens and other foreign compounds is information that is needed for the clinician and for judging the safety of various food additives, supplements, and other compounds to which man is exposed. In addition, tumors induced in non-human primates are likely to be more useful than the rodent tumor system now used to predict the usefulness of anticancer agents for man. In particular, the owl monkey may serve as a useful model for lymphoma and leukemia and for combination therapy with antiviral agents. Potential diagnostic tests for precancerous and malignant tissue may also be discovered by use of non-human primate tumors; few such tests now are useful for man. The usefulness of immunotherapy in the treatment of precancerous as well as frank neoplasia can be ascertained with the DENA induced precancerous and liver tumor.

Proposed Course:

Treatment with present carcinogens will be continued in the monkeys, and it is hoped that an observation period of at least ten years will give some basis for predicting which compounds are likely to be carcinogenic in man.

More owl monkeys will be inoculated with HVS and when disease is apparent treatment with known antileukemic and antiviral agents will be undertaken.

A continued effort will be made to assess the carcinogenic potential and long term toxicity of procarbazine and other antitumor agents being used for treatment of non-neoplastic disease.

Treatment of monkeys with liver tumors will be continued with various chemotherapeutic agents and by intraarterial infusion.

Attempts will be made to develop primate tumor models of colon cancer and stomach cancer. Potential biological markers for various types of tumors will be investigated.

Studies will continue on the correlation of the production of AFP in monkeys with the time of appearance of tumors and with histopathologic changes in the liver and in the tumors.

Studies of the metabolism of the various classes of carcinogens in various species will be continued. Such studies, it is hoped, will give more insight into susceptibility or refractoriness of primates compared to rodents.

Various monkeys will be utilized for comparative disposition studies and toxicological evaluation of anticancer, antiviral, and antimicrobial agents.

Publications:

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3. Ablashi, D. V., Loeb, W. F., Valerio, M. G., Adamson, R. H., Armstrong,G.R., Bennett, D. G., and Heine, U.: Malignant Lymphoma with Lymphocytic Leukemia in Owl Monkeys Induced by Herpesvirus saimiri. J. Natl. Cancer Inst. 47: 837-855, 1971.

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5. Adamson, R. H.: Carcinogenicity Studies with Procarbazine. In Proceeding of the Chemotherapy Conference on Procarbazine (Matulane: NSC-77213): Development and Application March 13, 1970. U.S. Department of Health, Education, and Welfare, P.H.S. Bethesda, Md., 1971, 29-33 pp.

6. Adamson, R. H., Ablashi, D. V., Armstrong, G. R., and Ellmore, N. W.: Effect of Cytosine Arabinoside, Adenine Arabinoside, Tilorone, and Rifamycin SV on Multiplication of Herpesvirus Saimiri In Vitro. Antimicrob. Agents and Chemotherapy. 1: 82-83, 1972.

7. Adamson, R. H.: Some Aspects of L-Asparaginase Toxicity in Animals and Man. International Symposium on L-Asparaginase - Colloques Internationaux du Centre National de la Recherche Scientifique. No. 197: 327-333, 1971.

This report was previously entitled "Carcinogenesis in Primates".

Serial No. NCI-3512
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemical Pharmacology of Newer Antitumor Agents and Model Compounds

Previous Serial Number: Same

Principal Investigators: Anthony M. Guarino and Vincent T. Oliverio

Other Investigators: Richard H. Adamson, Reginald L. Reagan, Robert Sponzo, and James Althaus

Cooperating Units: Laboratory of Toxicology, NCI

Man Years:

Total:	4.2
Professional:	2.2
Other:	2.0

Project Description:

Objectives:

To study the biochemistry and pharmacology of camptothecin, ICRF 159, and various nitrosoureas. To examine the metabolism, biliary excretion, and urinary excretion of certain antitumor agents and model compounds in rats and other species in order to better understand the mechanisms of drug metabolism and transport.

Methods Employed:

A. Camptothecin, sodium (NSC 100,880)

The standard assay techniques and methods for determining plasma binding were the same as reported in last year's annual report, with the exception that the present investigations also included gel filtration column chromatography.

B. ICRF 159 (NSC 129,943)

Prior to initiating fate and distribution studies on this very unusual antineoplastic agent, we attempted to develop analytic methodology to detect

this compound. While we were able to develop some thin-layer chromatographic methods for the parent compound, similar efforts to develop a gas chromatographic or spectrophotometric method for the analysis of this compound met with failure. The recent acquisition of the C^{14}-labeled compound has diminished the need for the latter two methodologies and hence further studies on this compound have been transferred to one of our new contractors, Dr. Mitoma at Stanford Research Institute.

C. Methodology on Nitrosoureas

Cyclohexyl and chloroethyl-^{14}C-MeCCNU were used in clinical pharmacologic studies in eight patients. MeCCNU in doses of 120 to 290 mg/m^2 were given orally and levels of radioactivity and parent compound were determined in plasma, urine, CSF, vomitus, and ascites. All samples were assayed for the presence of total radioactivity, intact drug, and decomposition products, using the techniques of thin-layer chromatography, combustion, and liquid scintillation counting. The L1210 cytotoxicity of MeCCNU in vitro was also studied. MeCCNU in plasma and buffered saline was assayed for L1210 cytotoxicity. Plasma samples from patients receiving MeCCNU orally were also assayed for L1210 killing effect.

D. Model Metabolism, Biliary Transport, and Urinary Transport Studies

Standard methods as described in last year's annual report and in previous publications from these laboratories have been used.

Major Findings:

A. Camptothecin, sodium

The differences in plasma protein binding were extended to include 24 species at two different camptothecin concentrations, 30 and 100 μg/ml. In the species of most interest to experimental chemotherapy, the human, monkey, rat, dog and mouse, the percent binding was 99, 98, 93, 79, and 74 respectively. The degree of binding was best correlated with albumin and β-lypoprotein plasma levels, but binding to purified protein fractions was highest for α-globulin and β-lypoprotein and lowest for albumin of eight purified fractions studied. Protein binding studied by means of Sephadex G-100 chromatograph revealed that human plasma binding was extensive while there was no significant binding demonstrated by this technique for any of the purified blood protein fractions.

The high binding of this drug to the plasma of the rat led us to study the biliary transport of this compound to see if transport could be altered by other drugs, especially those that might be used in combination chemotherapy. Over a 1,000-fold range of doses of camptothecin, the drug readily appeared in the bile and the bile to plasma ratios exceeded 300 in some cases. Probenecid, methotrexate and bilopaque inhibited the biliary transport but not the plasma binding of camptothecin.

B. ICRF 159

Two thin-layer chromatographic systems were developed using Silica gel G plates. The systems of $CHCl_3$-methanol (9:1) and ethyl acetate-isopropanol (1:1) gave R_f values of 0.49 and 0.77, respectively. After development in either of these systems a variety of standard spray reagents were tested and the only one which gave reasonable results consisted of ceric ammonium nitrate. Spraying the chromatograms with this reagent dissolved in 10% sulfuric acid and heating on a hot plate, gave a lavender spot where the compound was present.

C. Nitrosoureas

Significant plasma levels of radioactivity were detected in as early as 10 minutes with peak levels occurring at 3-6 hours. The plasma levels of isotope were prolonged with an average half-life of 33 hours. About 60% of the radioactivity given was excreted in the urine within 48 hours and of this more than 50% was excreted in the first 12 hours. CSF samples obtained at 2 and 3 hours, respectively, from two patients given cyclohexyl-labeled MeCCNU showed concentrations which were only 10 and 15 percent of concurrent plasma ^{14}C levels. Degradation of the drug is extremely rapid since no intact drug or parent compound was detected in any plasma, urine, CSF, or gastric sample.

The in vitro effect of MeCCNU on L1210 leukemia cells was also studied. When MeCCNU was added to plasma and incubated with L1210 cells in culture for 24 hours, a linear dose-killing effect relationship was seen. Plasma levels of 10 μg/ml or less did not significantly inhibit the growth of cells at 24 or at 48 hours. The lethal dose of MeCCNU for 50% of the cells exposed for 24 hours was at a concentration in plasma of 50 μg/ml. When the L1210 killing ability of MeCCNU in plasma was compared to MeCCNU in buffered saline, it was noted that when the drug was in plasma there was a 15-20% increase in killing activity at 24 hours. When plasma samples of patients receiving MeCCNU were added to L1210 cells in culture, no killing effect or inhibition of growth could be demonstrated.

D. Model Metabolism, Biliary Transport, and Urinary Transport Studies

1. Transport

a) Biliary excretion of glucuronides

Glucuronide conjugation is one of the most important ways by which the body rids itself of a variety of chemotherapeutic agents. Our studies reported last year, where the Gunn strain of rat, which has a congenital deficiency in the ability to synthesize certain glucuronides, have been extended to now include studies of the excretion of phenol red and phenol red glucuronide. Since this compound is widely used in test of renal function we wish to

investigate the role of the biliary and urinary systems in excreting the parent drug and its glucuronide.

b) Comparative pharmacology of biliary and urinary excretion of foreign compounds

It has been hypothesized that the biliary excretion of foreign compounds may have developed before renal excretion of such compounds in the evolutionary development of animals. The studies reported in last year's annual report wherein results were outlined for the biliary excretion of acidic, basic and neutral compounds in the dogfish, have been extended to include a greater number of compounds and also an experimental design which enables the simultaneous collection of bile and urine from the spiny dogfish (Squalus acanthias) For some of these compounds other species such as the hagfish and flounder have also been investigated. Listed below are the compounds studied to date along with the species in which the study has been conducted:

Phenol red	dogfish, flounder, hagfish
Camptothecin Na	dogfish
Probenecid	dogfish
Indocyanine green	hagfish, flounder, dogfish
Cyclophosphamide	dogfish, lobster
Procaine amide ethobromide (PAEB)	dogfish
Phenolphthalein	dogfish
Benzylpenicillin	dogfish
Tetracycline	dogfish
Rifamycin SV	dogfish
Procarbazine	dogfish
Methyl Hg	dogfish
DDT	dogfish, flounder
MTX	dogfish

The bile to plasma ratios for these compounds in the dogfish ranged from the lowest value of 8 for cyclophosphamide, while the highest 24 hour bile to plasma ratio found by us occurred in camptothecin and was 10,600.

c) Biliary excretion of demethylchlortetracycline

Compounds with the tetracyclic skeleton as part of their molecular configuration occur in both the antibiotic and antineoplastic drug classes. The availability of C^{14}-demethylchlortetracycline (DMCT) led us to study the biliary transport of this compound as a possible model for two tetracyclic antineoplastic agents, namely daunomycin and adriamycin. We found that in the renal ligated rat, this compound readily appeared into the bile and after a dose of 10 mg/kg the bile to plasma concentration ratio was 29. When tetracycline

and DMCT were simultaneously administered to rats the former compound markedly depressed the biliary excretion of demethylchlortetracycline. It appears that DMCT is excreted in part by a process of active transport and it is likely that daunomycin and adriamycin would be handled similarly.

d) Fate and distribution of DDT and Methyl Mercury in the lobster

We found that after administration of C^{14}-DDT or C^{14}-Methyl Mercury by either intravascular injection into the pericardial sinus, feeding contaminated food, or addition of the compounds to ambient water gave rise to uptake of these compounds in various organs of the lobster. The hepatopancreas was a dominant organ of storage for both compounds and the calculated $t_{\frac{1}{2}}$ for the disappearance of this compound was 46 days in the case of DDT and 13 days in the case of Methyl Mercury. There were peculiar organ redistributions for both of these compounds at later times, i.e. 3-4 weeks, where the redistribution appeared to occur largely from the hepatopancreas to skeletal muscle, in particular the tail muscle. Because of the possible carcinogenic, mutagenic, embryonic and teratogenic effects of these agents, it may be of considerable importance that we noted gonadal uptake of the compounds occurred only to a moderate extent but that these organs retained these levels for long periods of time, i.e. up to one month.

2. Conditions Altering the Metabolism of Drugs

a) Species differences

In an effort to further our knowledge of the species differences in responses which can be predicted from animals to humans, we have extended our studies on the drug metabolizing activity of microsomes and submicrosomal fractions in the guinea pig, monkey and rabbit. We had previously investigated the distribution of drug metabolizing components in smooth and rough microsomal membrane fractions of various mammalian livers, and have presently extended these studies to include further subfractionation of the smooth membranes in one of these species, i.e. the guinea pig. Hepatic microsomes were fractionated into three populations - rough, smooth I (S-I) and smooth II (S-II), by centrifugation in discontinuous sucrose gradients in the presence of Cesium^{++} and Mg^{++}. The distribution of various drug metabolizing components in the smooth membranes were 2-3 times higher than in rough membranes. Comparison of the two smooth membrane fractions revealed a statistically significant concentration of drug metabolizing components in the S-II microsomes. Thus ethylmorphine N demethylase, aniline hydroxylase, NADPH-cytochrome C reductase, and cytochrome P-450 were 20-30 percent higher in S-II than in S-I membranes. Pretreatment of guinea pigs with phenobarbital for four days increased the specific activities of ethylmorphine N

demethylase, aniline hydroxylase, NADPH-cytochrome C reductase and cytochrome P-450. The increase for each parameter was approximately the same magnitude in each of the three microsomal fractions.

3. Mass Spectral Identification Techniques of Drug Metabolites

As mentioned previously the formation of glucuronide conjugates of many drugs does occur. The methods usually used to identify such conjugates of drugs are lacking in both facility and in definitiveness. By the use of standard derivatizing techniques such as aceylation and by a combination of chemical ionization and electron impact mass spectrometry, we have initiated studies to identify four compounds and their corresponding glucuronides.

Significance to Bio-medical Research and the Program of the Institute:

The plasma protein binding studies completed for camptothecin are very important in light of the recent publications on theoretical changes which can occur in drug distribution from such binding. Calculations have shown that small changes in protein binding of drugs in plasma, as has been seen in a variety of species, could cause redistribution of highly bound drugs between tissue and plasma. Pharmacokinetic predictions suggest that redistribution would be the greatest after changes in tissue binding of highly bound drugs, hence camptothecin would fall under this category. These studies fit in well with other contract areas of the program where things such as degree of plasma protein binding are being sought as possible reasons for differences in the achieved plasma levels in patients given similar doses of the drug. The biliary excretion and drug metabolism studies involving varieties of species are important in providing a repertoire of species differences which we will be able to call upon when a specific pharmacologic problem arises. The studies of the lobster provide a method for the early assessment of adipose sequestering of lipid soluble antineoplastic agents, while in the case of Methyl Mercury where extensive binding occurs to SH groups may provide models for neoplastic agent binding in a similar manner. The microsomal studies are of value in understanding more precisely the mechanisms of inducing drug metabolism so such techniques can be applied rationally to drugs such as cytoxan, whose active metabolite is formed more readily after phenobarbital pretreatment.

Proposed Course:

1. Pharmacologic disposition studies of camptothecin are nearing completion and will be ready for publication in the near future.

2. Future studies on the excretion of metabolites into dogfish bile will be initiated for the more instable antineoplastic agents. The rational for this is that biotransformation usually occurs at a much slower rate in marine species than mammalian species. We feel that this slower rate of metabolism can be used to advantage in studying anticancer agents that are too rapidly metabolized in mammals.

3. Biliary transport and microsomal drug metabolism studies will continue on various antineoplastic agents and model drugs.

4. Much more extensive studies will be conducted using the combination of gas chromatography and mass spectroscopy in studying the metabolism of antineoplastic agents, such as the nitrosoureas.

Publications:

1. Oliverio, V. T. and Guarino, A. M.: Isolation and identification of drug metabolites: Isotope dilution analysis. In Gillette, J. R. and Brodie, B. B. (Eds.): Handbook of Experimental Pharmacology. Heidelberg, Germany, Springer-Verlag Co., 1971, pp. 160-177.

2. Guarino, A. M. and Fales, H. M.: Isolation and identification of drug metabolites: Gas chromatography-mass spectrometry. In Gillette, J. R. and Brodie, B. B. (Eds.): Handbook of Experimental Pharmacology. Heidelberg, Germany, Springer-Verlag Co., 1971, pp. 178-208.

3. Gram, T. E., Schroeder, D. H., Davis, D. C., Reagan, R. L., and Guarino, A. M.: Enzymic and biochemical composition of smooth and rough microsomal membranes from monkey, guinea pig and mouse liver. Biochem. Pharmacol. 20: 1371-1381, 1971.

4. Gram, T. E., Schroeder, D. H., Davis, D. C., Reagan, R. L., and Guarino, A. M.: Further studies on the submicrosomal distribution of drug-metabolizing components in liver. Localization in fractions of smooth microsomes. Biochem. Pharmacol. 20: 2885-2894, 1971.

5. Guarino, A. M., Helman, S. I., and Schanker, L. S.: Biliary transport of demethylchlortetracycline. Xenobiotica 1: 257-263, 1971.

6. Adamson, R. H. and Guarino, A. M.: The effect of foreign compounds on elasmobranchs and the effect of elasmobranchs on foreign compounds. Comparative Biochem. Physiol., in press.

7. Kinter, W. B., Merkens, L. S., Janicki, R. H., and Guarino, A. M.: Studies on the mechanism of toxicity of DDT and polychlorinated biphenyls (PCBs): Disruption of osmoregulation in marine fish. Environ. Health Perspective, in press.

Serial No. NCI-6108
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanisms of Action and Mechanisms of Resistance to Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Robert G. Gallo

Other Investigators: Vincent T. Oliverio, Vincent T. DeVita, Jacqueline Whang-Peng, and Hilton B. Levy

Cooperating Units: Human Tumor Cell Biology Branch and Medicine Branch, NCI and National Institute of Allergy and Infectious Diseases

Man Years:

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design new anticancer agents utilizing knowledge obtained so that a higher cell kill can be obtained and the problem of resistance can be circumvented.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis.

In addition, transfer RNA is prepared by standard techniques and iso-accepting species of t-RNA are isolated by use of a Freon reverse phase partition column.

Major Findings

This year we have been concerned with the mechanism of action of various interferon inducers, interferon per se, camptothecin, various nitrosoureas and hycanthone. In addition the control of aspargine synthetase and uptake of tRNA has received additional study.

Poly I·Poly C

Both poly I·poly C and interferon inhibited to a significant extent focus formation by Moloney Sarcoma Virus. In addition, poly I·poly C, mouse serum interferon, and tilorone all had similar antitumor activity against several transplanted tumors and all were not effective against another spectrum of tumors. Studies in animals and tissue culture show that poly I·poly C inhibits protein and RNA synthesis in tumor cells. The antitumor activity of poly I·poly C therefore is due to a superimposition of 3 effects; first, to a direct action of interferon which it induces; second, direct and selective chemotherapeutic action on the tumor by inhibition of macromolecule synthesis; and third, by enhancement of immunological rejection of foreign antigens.

Camptothecin

Camptothecin was active in increasing survival time in mice bearing various experimental leukemias. In addition, it produced 70-90% long-term survivors in mice bearing leukemias L5178Y or K1964. It was also active against the plasma cell tumor YPC-1 but less effective against a mast cell tumor and a reticulum cell sarcoma. The alkaloid was also cytotoxic in vitro against three cell lines. Studies using human lymphocytes stimulated by phytohemagglutinin (PHA) indicated that DNA synthesis was strongly inhibited with concentrations of the drug that did not affect RNA or protein synthesis. Camptothecin not only inhibited DNA synthesis but also caused a G_2 lesion which prevented subsequent mitosis. It did not have cytocidal effects on resting "G_o" lymphocytes. However, when "G_o" lymphocytes exposed to the alkaloid were subsequently stimulated to enter cycle, their division was still blocked. These observations not only imply that eventual cell proliferation is necessary for the antitumor activity of camptothecin, but also suggest that the drug may be useful for tumors with long G_1 intervals.

CCNU 1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea

Studies on the effects of CCNU and its degradation products on the duration of DNA synthesis in nitrosourea-sensitive and -resistant leukemia L1210 were performed and indicated that: the characteristic prolongation of DNA synthesis noted with the nitrosourea derivative 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was produced by CCNU; of the postulated decomposition

products of CCNU, only cyclohexylisocyanate produced a similar effect; and survival studies in sensitive and resistant strains of leukemia L1210 tend to link the prolongation of DNA synthesis by these drugs with their therapeutic efficacy, but improved survival in sensitive tumor strains was not produced by the degradation product cyclohexylisocyanate. The failure of cyclohexylisocyanate to prolong survival seems most likely related to a transient and reversible prolongation of DNA synthesis, perhaps due to pharmacologic differences between it and the parent compound, CCNU. The portion of the nitrosourea molecule from which the isocyanate moiety is derived seems to be implicated in part, in the antitumor activity of the nitrosoureas, perhaps by degradation after distribution into tumor tissue.

Hycanthone

Studies of the mechanism of the antitumor activity seen with hycanthone have recently began and preliminary studies indicate that hycanthone *per se* is the active moiety and that it intercalates with DNA and inhibits DNA synthesis.

Studies on Asparagine Synthetase

Our studies of the control of asparagine synthetase is summarized below.

Some L-asparaginase sensitive cells have an increase in tRNA acceptor activity for asparagine relative to that found in cells resistant to L-asparaginase.

L-asparaginase sensitive and resistant cells have at least 3 isoaccepting species (3 peaks) $tRNA^{Asn}$, at least two of these species are $tRNA^{Asn}$, the third species may be a mixture of $tRNA^{Asn}$ and $tRNA^{Asp}$.

Cells sensitive to L-asparaginase have a reduced peak 1 and a relative increase in peak 2 and peak 3 compared to the cells resistant to L-asparaginase.

L5178Y cells have a minor unique species (peak 4). The nature and significance of this peak is presently unknown but preliminary experiments suggest it may be glutamine. Further studies using L5178Y L-asparaginase resistant cells will aid in the study of peak 4. If this species is verified as glutaminyl-tRNA the glutaminyl-tRNA profiles of L5178Y and L1210 cells are likely to me markedly different, and it may implicate glutaminyl-tRNA in the regulation of asparagine synthetase.

Uptake of tRNA

Uptake of tRNA (*E. coli*) was demonstrated in murine leukemia, L1210, a human lymphoblast (NC-37) cell line, and fresh peripheral blood human lymphocytes. Uptake of tRNA was rapid, reaching a maximum within 45 seconds; was greater at 0°C than at 37°C; and was linear with concentrations up to approximately 50 µg/ml. Above 50 µg/ml the uptake was saturated. The tRNA was distributed in nuclei, lysosomes, mitochondria and cell "sap".

This uptake of exogenous tRNA apparently was not due to altered membrane permeability or impaired cell viability nor to ribonuclease degradation of the macromolecule. Furthermore, approximately 20% of the tRNA taken up by the cells remained functional and apparently intact. This was demonstrated by: acylation with E. coli aminoacyl-tRNA synthetases, methylation with leukemic cell tRNA methylases, and demonstrating [^{14}C]-labeled 4S RNA in the cytoplasmic fraction of the leukemic cells after the addition of [^{14}C]-E. coli tRNA. Results with phytohemagglutinin (PHA) stimulated lymphocytes suggested that uptake was enhanced when cells were synthesizing DNA. Studies on the mechanism of uptake suggest a complicated process involving both uptake and an active process of extrusion.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the mechanism of action of anticancer agents will lead to the design of newer drugs, hopefully, with greater cell kill and less toxicity. Knowledge of the mechanism of resistance to various anticancer agents will lead to measures that minimize development of resistance or will overcome this resistance. In addition, there is the possibility that resistant cells can be converted into sensitive cells.

Proposed Course:

The mechanism of antitumor activity of several newer anticancer agents, including hycanthone, coralyne, maytansine, gallium, and other metals will be investigated. Further studies with regard to mechanism of resistance to anticancer agents will also be undertaken.

Publications:

1. Gallo, R. C., Whang-Peng, J., and Adamson, R. H.: Studies on the Antitumor Activity, Mechanism of Action, and Cell Cycle Effects of Camptothecin. J. Natl. Cancer Inst. 46: 789-795, 1971.

2. Bray, D. A., DeVita, V. T., Adamson, R. H., and Oliverio, V. T.: Effects of 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU; NSC-79037) and its Degradation Products on Progression of L1210 Cells Through the Cell Cycle. Cancer Chemo. Repts. 55: 215-220, 1971.

3. Levy, H. B., Adamson, R., Carbone, P., DeVita, V., Gazdar, A., Rhim, J., Weinstein, A., and Riley, F.: Studies on the Antitumor Action of Poly I:Poly C. In: Biological Effects of Polynucleotides. Edited by R. F. Beers and W. Braun, Springer-Verlag, 1971 pp. 55-65.

4. Gallo, R. C., and Adamson, R. H.: Asparagine, Asparaginase, and tRNA. In: Symposium International Sur La L-Asparaginase, Colleque Internationaux C.N.R.S. #197, Editions Du Centre National De La Recherche Scientifique, Paris, France, 1971 pp. 121-132.

5. Herrera, F., Adamson, R. H., and Gallo, R. C.: Transfer RNA as an Informative Molecule and Its Uptake by Normal and Leukemic Human and Murine Leukocytes. In: Informative Molecules in Biological Systems, edited by L. G. H. Ledoux, North-Holland Publishing Company, Amsterdam, London, 1971 pp. 377-397.

Serial No. NCI-6116 (new)
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Nitroreductase Activity of Hepatic Aldehyde Oxidase

Previous Serial Number: None

Principal Investigator: David G. Johns

Other Investigators: Mary K. Wolpert

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives:

A number of nitro-substituted N-heterocyclic compounds are active carcinogens; their carcinogenic activity is thought to be due however to the corresponding hydroxyamino metabolite, rather than to the parent nitro compound. Most mammalian nitroreductase activity is found in the liver, and occurs in both microsomal and soluble supernatant fractions from liver homogenates. The soluble supernatant nitroreductases (prominent among which are aldehyde oxidase, xanthine oxidase, and DT-diaphorase) are able to catalyze reduction to the biologically active hydroxyamino stage, while the microsomal nitroreductases appear to be able to carry the reduction further, to the corresponding amino compounds. The objective of this project is to characterize the previously little studied nitroreductase activity of mammalian hepatic aldehyde oxidase, and to assess its quantitative significance in the biological activation of this group of carcinogens.

Methods Employed:

Hepatic aldehyde oxidase is obtained from a number of mammalian species (rabbit, guinea pig, mouse, and rat) and purified by ammonium sulfate fractionation followed by gel filtration, column chromatography, and other standard enzyme purification methods. Nitroreductase activity is assayed spectrophotometrically under anaerobic conditions, using either nitrofurazone

or the carcinogen 4-nitroquinoline-N-oxide as electron acceptor. The products of enzymic reduction are identified by ultraviolet and infra-red absorption spectroscopy, by thin-layer and paper chromatography, and by carbon-nitrogen-hydrogen combustion analysis. Reference compounds are prepared by chemical reduction of the parent nitro compound.

Major Findings:

Nitrofurazone, 2-bromo-5-nitrothiazole, and the carcinogen 4-nitroquinoline-N-oxide were readily reduced by aldehyde oxidase under anaerobic conditions to the corresponding hydroxylamino compounds; a variety of other nitro compounds, including p-nitrobenzoate, did not show detectable acceptor activity. Effective electron donors for the reduction included aldehyde substrates such as butyraldehyde and benzaldehyde, and N-heterocyclic substrates such as 2-hydroxypyrimidine, 3'5'-dichloromethotrexate, and N-methylnicotinamide. NADH and NADPH failed to show detectable electron donor activity. The nitroreductase activity of aldehyde oxidase was significantly inhibited (>50%) by menadione (15 μM) and by Triton X-100 (7×10^{-4}%); in contrast to that of xanthine oxidase, however, it was not inhibited by 4-hydroxypyrazolo(3,4-d)pyrimidine (allopurinol) (5 μM). The ratios of aldehyde oxidase:xanthine oxidase nitroreductase activity in the 105,000 x g supernatant fraction of liver homogenates from several mammalian species, utilizing nitrofurazone as electron acceptor, were as follows: rabbit: 4.8:1; guinea pig: 1.4:1; and rat: 0.9:1.

Significance to Bio-medical Research and the Program of the Institute:

Nitro compounds are widely distributed in the environment, and are readily reduced in vivo to hydroxyamino, nitroso, and amino derivatives. The reduced forms of certain nitro compounds are known to possess activity as carcinogens. Study of the nitroreductase activity of aldehyde oxidase and the assessment of its quantitative significance in nitro group reduction in vivo can thus lead to information of potential relevance to the etiology of neoplastic disease.

Proposed Course:

While our present studies show that hepatic aldehyde oxidase possesses significant nitroreductase activity in vitro, the quantitative role of this reaction in vivo has not yet been determined. Attempts will be made to study the reaction under conditions more nearly approaching those obtaining in vivo, and the effect of specific aldehyde oxidase inhibitors on nitro group reduction and on the carcinogenic activity on nitroquinoline-N-oxide in the whole animal will be carried out.

Publications:

Wolpert, M. K. and Johns, D. G.: Nitroreductase activity of mammalian hepatic aldehyde oxidase. Fed. Proc. 31: 606, 1972.

Serial No. NCI-6120 (new)
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Kinetic Profile of Drug Action In Vivo

Previous Serial Number: None

Principal Investigators: Bruce A. Chabner and Robert C. Young

Other Investigators: Charles B. Myers and Steven Rosenoff

Cooperating Units: Solid Tumor Service, Medicine Branch, NCI

Man Years:

Total: 2

Objectives:

In using any pharmacologic agent, certain critical information is needed in order to design an optimal schedule of drug administration. One must know:

1) The nature and kinetics of distribution and disposition of the agent.

2) Its mechanism of action.

3) The levels of parent drug or actor metabolite required for the desired effect on target tissue.

While extensive information is now accumulating with regard to pharmacokinetics and mechanism of action of the common antitumor drugs, little is known about the drug concentrations which produce toxicity and antitumor effects in vivo. This important consideration is the object of our research efforts. We have chosen methotrexate as a model agent for study because it is easily measured and active as the parent compound. Secondly, its mechanism of action, the inhibition of dihydrofolate reductase, with consequent block of the conversion of dUMP to dTMP, allows one to follow it action in vivo by determining the incorporation of ^{3}H-UdR into DNA.

Methods Employed:

Methotrexate (MTX) levels were determined by the standard dihydrofolate-reductase inhibition assay, which allows quantitation of MTX levels of 10^{-9}M or greater. BDF_1 and CDF_1 mice, some bearing L1210 ascites tumor, were injected with 5, 50, or 350 µg/kg MTX and were sacrificed at frequent intervals. One hour prior to sacrifice ^{3}H-UdR was injected i.p. in tracer amounts and the incorporation of label into DNA of bone marrow, intestinal epithelium, and ascites tumor was determined by phenol extraction and scintillation counting.

Major Findings:

1. The extended pharmacokinetics of methotrexate in mice have been defined using the highly specific dihydrofolate reductase assay. The persistence of MTX in plasma for several days at concentrations of 10^{-8}M or greater has been demonstrated following doses of 350 mg/kg (LD_{30}) or greater.

2. At all dose levels, >50% inhibition of ^{3}H-UdR incorporation into DNA was observed in all tissues when plasma levels were 10^{-8}M or greater, while rapid recovery of this biochemical function took place when plasma [MTX] fell below this figure.

3. The recovery of bone marrow clearly antedated recovery of intestinal epithelium at the two higher dose levels of the drug, thus explaining the dose limiting nature of intestinal toxicity of MTX in mice.

4. A marked overshoot in ^{3}H-UdR incorporation was observed during the recovery period following MTX; this overshoot in DNA synthesis is likely to be associated with marked sensitivity to repeat doses of the agent at this time.

In summary, these studies have shown that each dose level of MTX is associated with a characteristic kinetic profile of biochemical effect which is intimately related to the pharmacokinetics of the drug. In particular, inhibition of ^{3}H-UdR incorporation into DNA was maintained so long as plasma levels of MTX remained above 10^{-8}M.

Proposed Course:

1. Determine threshold concentration of drug required for inhibition of DNA synthesis in various tumors in mice.

2. Exploit observed differences in recovery of host and tumor in the design of less toxic and more effective schedules of drug administration.

3. Extend studies to man, to determine sensitivity of human neoplasms and bone marrow to MTX.

4. Begin similar studies with 5-FU.

Publications:

None

Serial No. NCI-6121 (new)
1. Lab. of Chemical Pharmacology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Purification and Properties of Carboxypeptidase G_1

Principal Investigators: Bruce A. Chabner

Other Investigators: Joseph R. Bertino, David Makula, Paul L. Chello and David G. Johns

Cooperating Units: Department of Pharmacology, Yale University School of Medicine

Objectives:

1. To simplify and improve the purification of carboxypeptidase G_1 (CPD G_1) in order to allow large scale production of this enzyme.

2. To determine the antitumor activity of CPD G_1 in mice.

3. To determine the toxicity of CPD G_1 in mice, dogs, and monkeys.

4. To begin clinical trials of this agent if warranted by animal data.

5. To investigate prevention of methotrexate toxicity by CPD G_1.

Results:

CPD G_1, a folate-cleaving enzyme from Pseudomonas stutzeri, has been purified to homogeneity by a series of ion exchange, gel filtration, and electrofocusing steps. An improved, large scale procedure utilizing batch wise separation with an ion exchange resin SP-Sephadex is currently under study and promises to be successful in allowing the production of $5 \times 10^5 - 10^6$ units of enzyme in a single lot, as required for clinical investigations.

Preliminary studies have indicated a small, but significant antitumor effect against L1210, L5178Y, and Sarcoma 180 (ILS: 15-30%), and a highly significant effect against Walker 256 (ILS: 43-89%), a methotrexate resistant tumor. In vitro studies demonstrated that CPD G_1 does not selectively inhibit thymidylate synthesis, as does methotrexate, but depresses DNA synthesis via other as yet undefined mechanisms.

No acute or chronic toxicity has been observed in mice and dogs given folate-depleting doses of purified CPD G_1 in single or multiple dose schedules, despite clear cut folate depletion. In particular, antibody production versus CPD G_1 was looked for, but using a highly sensitive radio

immuno-assay, has been found thus far only in rabbits given the enzyme in Freund's adjuvant. Acute folic acid depletion also appears to have no adverse effect on the hematopoeitic systems of mice and dogs in the doses used.

Other potential uses of CPD G_1 have been explored. The response of hepatic folate levels to acute plasma folate depletion has been studied. Hepatic folate levels fall abruptly in mice following CPD G_1; a secondary rise and fall in folates is observed 12-18 hrs after administration, possibly representing deconjugation and release of hepatic polyglutamates.

Also it has been observed that CPD G_1 is capable of hydrolyzing extracellular methotrexate. We have studied the effectiveness of CPD G_1 as an instrument for preventing MTX toxicity by administration of the enzyme at intervals following methotrexate. It was found that CPD G_1 is capable of partially preventing the lethal toxicity of 1000 mg/kg MTX, an LD_{80} level of the drug, when given 24 hrs after MTX. A second dose of CPD G_1 given 48 hrs after MTX furnishes complete protection against MTX toxicity. The survival of mice with L1210 leukemia treated with high dose MTX and CPD G_1 rescue approximated that of animals treated with MTX-leucovorin, and was better than that obtainable with the highest tolerated doses of MTX without protection. CPD G_1 rescue may have several advantages over leucovorin:

1) CPD G_1 does not reverse the established intracellular blockade of thymidylate synthesis, as does leucovorin.

2) CPD G_1 has antitumor effects of its own.

3) Persistence of leucovorin in plasma and tissues makes the use of subsequent doses of MTX ineffective, while CPD G_1 is quickly inactivated in plasma at 37° (1/2 life 6-8 hrs).

In addition to demonstrating the feasibility of degrading drug *in vivo* with administered enzyme, these studies have illustrated the importance of the slow third phase of methotrexate disposition in producing lethal toxicity in mice.

Publications:

1. Chabner, B. A. and Bertino, J. R.: Activation and inhibition of carboxypeptidase G_1 by divalent cations. *Biochim. Biophys. Acta*, in press.

2. Chabner, B. A., Chello, P. and Bertino, J. R.: Antitumor activity of a folate-cleaving enzyme, carboxypeptidase G_1. *Cancer Research*, in press.

3. McCullough, J. L., Chabner, B. A. and Bertino, J. R.: Purification and properties of carboxypeptidase G_1. J. Biol. Chem. 246:7027-7213, 1971.

4. Bertino, J. R., Levitt, M., McCullough, J. L. and Chabner, B. A.: New approaches to chemotherapy with folate antagonists: Use of leucovorin "rescue" and enzymic folate depletion. Ann. N.Y. Acad. Sci. 186: 486-495, 1971.

SUMMARY REPORT

LABORATORY OF MOLECULAR PHARMACOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 -- June 30, 1972

Many anti-tumor agents appear to have primary effects on nucleic acid metabolism. Current work in this laboratory is concerned with drug actions on nucleic acids, ranging from the reaction of drugs with DNA (or related targets) to the production of selective cytotoxicity. These studies have concerned (1) the nature of complex formation between drug and DNA, (2) effects on the intracellular structure of DNA and RNA, (3) drug-induced changes in DNA function, as indicated by changes in RNA synthesis patterns, and (4) drug effects on the relation between DNA and nuclear protein synthesis. We feel that studies in these areas are likely to yield clues regarding the origin of selective cytotoxicity.

Reaction of Anthramycin with DNA.

We have continued an intensive interest in anthramycin, our previous work having indicated that the degree of specificity and irreversibility of the binding of anthramycin to DNA is unique for a small molecule. The drug is chemically unrelated to other known anti-tumor agents, and it has none of the chemical features previously known to produce tight binding to DNA. In addition to the potential chemotherapeutic applicability of this new mode of reaction with DNA, anthramycin may find application as a particularly effective probe of DNA conformation.

Our work during the past year has yielded the following new information on the reaction of anthramycin with DNA: (1) the reaction requires the presence of guanine residues in a deoxypolynucleotide; (2) there is a linear competition with actinomycin for binding sites; (3) the kinetics of the reaction are second-order, but limited by neighborhood exclusion, (4) circular dichroism spectra indicate that the phenolic group of anthramycin participates in the binding; (5) electric dichroism measurements indicate that the plane of the anthramycin molecule is approximately perpendicular to the plane of the bases; (6) viscosity, sedimentation, and light scattering measurements over a range of DNA molecular weights indicate that the effect of anthramycin binding on DNA is to increase the stiffness of the DNA helix without increasing its length.

To our knowledge, the properties disclosed under (5) and (6) above have not previously been reported for any DNA-drug complex. Thus, the anthramycin-DNA complex appears to fall into a new category.

Measurement of DNA Single-strand Breaks in Cells.

Several anti-tumor agents have been found to produce single-strand breaks in DNA, and the ability to repair these breaks has been suggested as a

determinant of cell survival. The current techniques for the measurement of single-strand breaks, however, are not easily applicable to extensive biological studies. We have developed a new method that we believe will be more suitable for these studies.

Effects of Drugs on RNA Metabolism in L1210 Cells.

Compounds that bind to DNA in the cell generally cause an inhibition or perturbation of RNA synthesis. We have obtained evidence that the pattern of effects on RNA synthesis depends on the nature of the binding to DNA, especially, we think, on the degree of reversibility of the complex. During the course of this work (much of which was outlined in last year's annual report), it was noted as an incidental finding that certain drugs inhibit the maturation processing of nucleolar 45s RNA. This effect, however, correlated with the type of effect produced on RNA synthesis. We have therefore continued to study effects on RNA processing, even though it does not necessarily reflect a direct DNA function.

During the past year, further studies have been carried out with nitrosoureas and with camptothecin. A structure-activity study of the inhibition of nucleolar RNA processing by nitrosoureas showed requirements for the 1-chloroethyl and 1-nitroso groups. The chloroethyl-nitroso half of the molecule, however, may not itself be the reactive portion, since the effect was also produced by isocyanates of the type expected to be formed from the other half of the molecule upon cleavage of the urea C-N1 bond. An important function of the chloroethyl-nitroso- part of the molecule may therefore be to generate isocyanates at the proper time and place in the cell.

The effects of camptothecin on DNA synthesis had previously been found to resemble those of the irreversible DNA-binders, anthramycin and nitrogen mustard. In particular, all three compounds caused a reduction of RNA chain-length, as measured by short uridine-incorporation times. This was puzzling because camptothecin does not bind to DNA in vitro. We now find that camptothecin differs from anthramycin and HN2 in that longer incorporation periods give RNA size distributions that approach normal, and in that, upon removal of drug RNA synthesis reverts to normal.

Many of our drug studies are based on measurement of RNA molecular weight distributions. These measurements, however, are subject to uncertainties arising from the tendency of some types of RNA to form aggregates and from the possibility of hidden chain breaks within helical segments. This problem was attacked by reducing the RNA chain interactions by means of formaldehyde. But extensive treatment tended to introduce new breaks. Detailed study of this problem has now produced two important results: (1) drug effects on RNA molecular weight distributions can be quantitated despite the introduction of a modest number of random breaks by formaldehyde treatment; (2) evidence was obtained for the existence of hidden chain breaks in nucleoplasmic RNA, but not in nucleolar RNA, of L1210 cells.

Helical Content of RNA from L1210 Cells.

Our finding that nucleolar RNA processing is inhibited by intercalating agents

suggested the possibility that the ribosomal RNA precursors may have helical segments at or near the processing sites; such segments could be preferential targets for the binding of intercalators. It seemed possible that such helical segments might be extensive enough to show an increased helical RNA content by the ribonuclease-resistance assay. Experiment, however, failed to disclose any such increase. However, the following significant results emerged from this work: (1) the ribonuclease-resistance assay was improved to make it suitable for the measurement of the short and imperfect helical segments expected in RNA from eukaryotic cells; in line with this expectation, RNA from L1210 cells was found to differ from double-stranded viral RNA in that the extent of ribonuclease resistance did not level off with increasing NaCl concentration. (2) Nucleoplasmic RNA of L1210 cells exhibited a greater extent of ribonuclease resistance than did either nucleolar or ribosomal RNA; (3) several attempts failed to confirm the report by others of an increase in ribonuclease resistance of RNA synthesized in the presence of actinomycin.

Effects of Chemotherapeutic Agents on Nuclear Proteins in L1210 Cells.

The method developed in our laboratory for fractionation of nuclear proteins by ion-exchange chromatography in the presence of urea was further characterized by amino acid analysis and gel electrophoresis of the peaks. The distribution of histone and non-histone protein species in the chromatographic peaks was thereby determined. The method was applied to the drug, cyclohexyl-chloroethyl-nitrosourea (CCNU). The drug caused changes in the protein patterns, the exact nature of which will require further study. The cyclohexyl moeity of CCNU was found to bind to several protein species recovered from nuclei. Studies with radioactive precursors indicated that CCNU affects the uptake of thymidine and valine into cells, perhaps reflecting an action on the cell membrane.

Studies on the Effects of Chemotherapeutic Agents on the Coordinate Synthesis of DNA and Nuclear Proteins.

The cellular synthesis of DNA and nuclear proteins is normally coordinated so as to maintain a balance between these associated components of chromatin. It seemed possible that this balance may be a crucial factor in the ability of a given cell type to survive challenge with a metabolic inhibitor. Thus, the survival of a cell treated with a specific inhibitor of DNA synthesis might depend on the ability of that cell to turn off the synthesis of nuclear proteins, so as to avoid an abnormal and perhaps lethal accumulation nuclear proteins.

As a first step, a variety of anti-tumor agents were surveyed to see how the turn-off of DNA, histone, and nuclear non-histone protein synthesis are related in L1210 cells. These cells, upon total inhibition of DNA synthesis by hydroxyurea, were found to continue synthesizing nuclear proteins initially at about half the normal rate, then tapering off to give a net accumulation of about 15% extra histone and non-histone proteins in the nuclei. The significance of this finding in relation to cell lethality remains to be determined by comparing different cell types having different sensitivities to hydroxyurea. A wide variety of other anti-tumor drugs were examined to determine whether any of them uncouple the syntheses of DNA and nuclear

proteins. Although no example of complete uncoupling was found, several partial effects were noted that may be worthy of further study.

Serial No. NCI-6103
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Chemotherapeutic Agents on L1210 Cell Nuclear Proteins

Previous Serial Number: Same

Principal Investigators: Vincent H. Bono, Jr., Paul V. Woolley, and Robert Dion

Other Investigators: None

Cooperating Units: None

Man Years

Total:	3
Professional:	3
Other:	0

Project Description:

Objectives:

This project is concerned with the development of techniques for the analysis of drug effects on cell nuclear proteins and with their application to drugs having both primary and secondary effects on synthesis of function of nuclear proteins.

Methods Employed:

L1210 cells were grown in suspension cultures. Proteins were labeled by allowing growing cells to incorporate carbon-14 and/or tritium labeled valine and/or tryptophan. Cell nuclei were isolated by homogenization in a non-ionic detergent and centrifuged through sucrose. Nuclear proteins were fractionated by their acid solubility characteristics and also by methods to be described under major findings. Nuclear DNA and associated protein was obtained by centrifugation through NaCl-urea. Isotopes were measured by standard methods of liquid scintillation counting.

Major Findings:

1) Analysis of nuclear proteins by ion-exchange chromatography in 6M urea.

The characterization of a new method for analysis of nuclear proteins by ion-exchange chromatography in 6M urea which was begun last year, was completed

during the current project year. We have previously reported the details of the preparation of radio-labeled whole nuclear protein from L1210 leukemia cells and its separation into a run-off and 6 protein peaks by ion-exchange chromatography. The first four peaks (A, B, C, and D) contain the histone proteins along with some non-histone protein. The run-off and peaks E and F are non-histone proteins.

During the current year the amino-acid analysis, and polacrylamide gel electrophoresis of these peaks were performed and the following conclusions were drawn:

1. The run-off protein contains a large number of acidic non-histone proteins but no histone proteins.

2. Peak A is a mixture of histones f2a1, f2a2, f3, and small amounts of non-histone protein.

3. Peak B is also a mixture of histones f2a1, f2a2, and f3 but is essentially free of non-histone protein.

4. Peak C is histone f2b with small amounts of f2a1.

5. Peak C is primarily histone f1 but also contains several non-histone proteins and small amounts of f2a1.

6. Peaks E and F are non-histone proteins.

This order of elution of histone proteins is the reverse of that obtained by chromatography in the absence of urea. It was recently reported that the salt elution order of histones from native calf thymus chromatin is also reversed in the presence of urea to give a pattern similar to that seen in this ion-exchange chromatography. This suggests that the effect of urea is on some characteristics of the histone proteins themselves rather than on the DNA to which they are bound. The major advantages of this urea ion-exchange chromatography are that the various proteins are separated without exposure to acid conditions and that all of the nuclear proteins are subjected to chromatography without prior separation into subfractions.

2) Effects of CCNU on nuclear proteins.

Studies on the effects of CCNU on the nuclear proteins of the L1210 leukemia cell were continued. Previously we found that 10^{-3}M CCNU caused the binding of cytoplasmic protein to the nucleus in amounts which increased with time of exposure (from 5% at 10 minutes to 50% at 2 hours). However, under these conditions there was no convincing evidence for an increase in the amount of protein tightly bound to DNA.

During the current year the effect of CCNU on the chromatography (ion-exchange in urea) of nuclear proteins was examined. A 10 minute exposure to 10^{-3}M CCNU resulted in a binding of 3% of cytoplasmic protein to the nucleus

(as seen before). The chromatographic behavior of the nuclear proteins indicated either a selective loss of histone from peaks B and C or else a selective gain in protein in run-off fraction and in peaks A and D. A 20 minute exposure to $5x10^{-4}$M CCNU (conditions which do not cause cytoplasmic contamination) indicated a decrease in peak B protein and an increase in run-off and peak A protein.

^{14}C-cyclohexyl labeled CCNU was studied under similar conditions and the following % distribution of label was found:

CCNU	R.O.	A	B	C	D	E	F
1 mM	80	6	4	3	3	1	3
0.5 mM	56	11	5	6	8	3	11

At 1 mM CCNU was 5 times as much ^{14}C label bound as did at 0.5 mM CCNU. There were several characteristics of elution that are not evident in the chart: the isotope in R.O. eluted slightly later than the main protein peak itself, the isotope in A is a tail on the R.O. peak and isotope in F is the only other sharp peak of ^{14}C-cyclohexyl label. Although it was anticipated that CCNU would carbamylate the histone proteins primarily, these findings appear to indicate the opposite (i.e. that it has reacted with non-histone proteins primarily).

At 5 to 10 µM CCNU the proliferation rate of L1210 leukemia cells was reduced to 50% control. 1 µM had no effect on proliferation rate and 20 µM completely inhibited proliferation but did not cause loss of a trypan blue exclusion function after 24 hours. At 100 µM, after a lag of two hours, cells became trypan blue positive with a half time of 1 hour and in the trypan blue negative cells, large single blebs were seen.

At 10 µM CCNU there is a rapid drop in both protein and DNA synthesis by 30-40% of control to 30 minutes. However protein synthesis recovers to 60% of control by 3 hours while DNA synthesis continues to fall slowly to 20% of control. Of interest, although acid soluble ^{14}C-valine drops (relative to control) and recovers just as protein synthesis does, acid soluble ^{3}H-thymidine rose to above control levels at 30 min. and remained up for 2 hours. This suggests (only tentatively) that protein synthesis may drop due to a decrease in precursor amino acid availability while DNA syntheses drops for reasons which result in an accumulation of precursor nucleotides.

These studies with CCNU indicate the possibility of at least a dual action, one on the cell membrane and the other on DNA synthesis. The studies with ^{14}C-cyclohexyl labeled CCNU and with labeled nuclear proteins were performed at concentrations (500 and 1000 µM) much above those needed for biological activity. The results at these high concentrations indicate that CCNU causes a small reduction of peak B proteins (f2a1, f2a2, and f3) and that the ^{14}cyclohexyl grouping binds to proteins that elute in both the run-off fraction and peak F (non-histone proteins). Similar studies should be done at 10 µM CCNU but these studies are limited by the specific activity of the

labeled CCNU and the precision of measurement of labeled nuclear proteins.

3) Screen for selective drug effects on the synthesis of nuclear proteins.

In view of observations that indicate a close coordinate control of DNA and histone protein synthesis, a biochemical screen was developed to screen for drugs that may alter this coordinate control. The experimental design was set to detect two types of effects: (a) a selective inhibition of histone synthesis (relative to non-histone nuclear protein and cytoplasmic protein) and (b) interference with the shut down of histone synthesis that occurs after totally inhibiting DNA synthesis with hydroxyurea. This was done by measuring the rate of synthesis of DNA (using ^{3}H-TdR), whole cell protein, nuclear non-histone protein and histone protein (using ^{14}C-valine) over a 3 hour period of drug exposure. Drug concentrations were chosen so as to reduce the rate of synthesis of one of these macromolecular fractions to less than 80% of control or reduce the average 24 hour proliferation rate to less than 50% of control.

A total of 23 drugs were studied. Of these, seven drugs met the criteria of selective inhibitors of histone synthesis: hydroxyurea, FUDR, quanazole, cytosine arabinoside, 3'-deoxyadenosine, daunomycin and anthramycin. However these are all considered to be false positives since they are known to directly inhibit DNA synthesis which secondarily causes a selective shut down of histone synthesis. The negative drugs were actinomycin D, nitrogen mustard, cycloheximide, emetine, thialysine, mitomalcin, dichlorodiamino platinum, tri-ornithine, spermine, calf thymus histone, phleomycin and deoxycytidine. The results of the screen for compounds that interfere with the shut down of histone synthesis produced by hydroxyurea was entirely negative.

Since histone protein lacks tryptophan, the effect of tryptophan deficiency on the synthesis of nuclear protein was examined. The rate of DNA synthesis dropped most rapidly to 35% of control during the fourth hour of deficiency. Histone synthesis fell faster than non-histone or whole protein synthesis, to 60% of control. These results indicate (a) that DNA synthesis is very sensitive to tryptophan deficiency and (b) that the secondary shut down of histone synthesis operated quite well in the tryptophan deficient state.

During the course of this drug screen, several observations of interest were made 1) Inhibition of RNA synthesis selectively with actinomycin D results in a reduction of histone synthesis rate with a T 1/2 of 3 to 4 hours. Inhibition of DNA synthesis selectively with hydroxyurea results in a biphasic reduction of histone synthesis rate with an early T 1/2 of 1 hour followed by a later T 1/2 of 2 hours. Total inhibition of RNA and DNA synthesis caused a prompt fall in histone synthesis rate with a T 1/2 of 0.5 hours. 2) Actinomycin D and anthramycin both selectively inhibit nuclear protein synthesis to a greater extent than whole cell protein synthesis. During a two hour exposure to 0.1 μg/ml, actinomycin D nuclear protein synthesis was reduced to 74% of control while cytoplasmic protein synthesis was 95% of control.

3) Mitomalcin at 25 and 50 µg/ml, while reducing DNA synthesis to 75% of control over a 3 hour exposure, had no inhibitory effect on histone synthesis. This was unlike two other drugs neocarzenostatin and spermine which in addition to reducing DNA synthesis to a comparable extent also reduced histone synthesis to 80 to 85% of control. This result suggests that mitomalcin may interfere with the secondary shut down of DNA synthesis. However, it did not prevent the shut down of histone synthesis induced by hydroxyurea. 4) Both dCR (2.5 mM) and pIpC (50 µg/ml) stimulate the proliferation rate but do not increase the final plateau level of L1210 cell growth. During the first 3 hours of exposure to dCR there is a stimulation of histone synthesis with no change in the synthesis of either non-histone or whole cell protein. 5) While extraction of histone protein from isolated nuclei with 0.15N H_2SO_4 extracts some non-histone protein (10-15% of the nuclear tryptophan) it was found that 0.25N HCl will extract considerably larger quantities of non-histone protein (30-35% of nuclear tryptophan). This latter extraction method has been used by many laboratories to produce a "histone" protein fraction.

Proposed Course and Relevance to the Program:

(1) From data in the published literature it is becoming increasingly clear that interruption of DNA synthesis in a proliferating cell does not always result in cell death. Certain cells (e.g. HeLa and mouse L-cells can survive prolonged periods of interrupted DNA synthesis without lethal consequences. Whereas other cells (e.g. CHO and L1210 leukemia cells) are lethally affected. In addition there is evidence that cells stimulated into cell division, (e.g. isoproterinol stimulated salivary gland, folic acid stimulated kidney, hepatectomized liver, and croton oil stimulated epithelium) are not lethally affected by interruption of DNA synthesis. In contrast normally proliferating cells (jejunum, tongue epithelium, erythropoetic cells and germinal follical cells of lymph nodes) are lethally affected.

Histone synthesis is temporarily related to DNA synthesis and the literature shows that the cellular response to interruption of DNA synthesis may vary from a prompt shut off of histone synthesis (HeLa cells) to a very prolonged continuation of histone synthesis (physarum polycephalum). It seems reasonable to ask then whether the survival or death of a cell in response to interrupted DNA synthesis can be correlated with the kind of control the cell exerts on histone synthesis.

With this in mind we plan to measure certain responses of cells to interruption of DNA synthesis by such drugs as hydroxyurea, cytosine arabinoside and FUDR. The response measurement that will be considered are (1) the uptake of new histone by the cell nucleus and some measure of its binding characteristics (e.g. salt dissociability relative to preformed histone) (2) the stability of preformed histones, (3) the cytoplasmic accumulation of histones (if any) (4) the potential of cytoplasmic ribosomes for the synthesis of histones and (5) the presence of messenger RNA associated with histone synthesis. Some or all of these responses will be taken as characterizing the cell response to interruption of DNA synthesis.

Cell types and conditions of drug exposure will be selected so as to allow studies to be made in cells which will survive as well as those which are lethally effected. Survival will be determined by methods such as plating efficiences or by mitotic collection. The practical aspect of this type of research is that if a more complete understanding of the relationship between DNA synthesis inhibition and cell death was available then it might suggest new ways to attempt to modify the host so as to increase the host tissue resistance to the cytotoxic effects of DNA synthesis inhibition. Conversely it might indicate how to increase the lethal effects on tumors which may have the capacity to survive interruption of DNA synthesis. In addition, an empirical screen for a selective inhibitor of histone synthesis or an agent that interfers with shut down control of histone synthesis, might produce just the modifying characteristics described above.

(2) (a) Continue studies on the strong binding of chemotherapeutic agents (such as nitrosoureas) to nuclear proteins in an effort to demonstrate selectivity of interaction. (b) Explore the possible effects of these drugs on nuclear bound enzymes such as phosphokinase activity. (c) Explore the effects of non-histone proteins on the conformation of DNA and nucleohistone, and the effects of non-histone protein enzymatic activity on such conformation. (d) Explore the possible value of fluoresence quenching of tryptophan as a measure of non-histone protein binding interactions with DNA during dissociation and/or reassociation of chromatin.

Publications: None

Serial No. NCI-6104
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Chemotherapeutic Agents on RNA Metabolism

Previous Serial Number: Same

Principal Investigators: Herbert E. Kann, Zafar Iqbal, David Gullion, and Kurt W. Kohn

Other Investigators: None

Cooperating Units: R. Regan, Laboratory of Chemical Pharmacology, NCI, (electron microscopy).

Man Years

Total:	4.5
Professional:	3.5
Other	1

Project Description:

Objectives:

To examine a variety of chemotherapeutic agents, known to interfere with RNA synthesis, for selective effects on the patterns of RNA synthesis and processing. This information may help to determine the mechanisms of action of these agents and to classify these agents according to the effects produced. In addition, this study aims to utilize specific drug actions to elucidate the mechanisms of RNA synthesis and processing in the cell nucleus.

Methods Employed:

1) Growth of L1210 cells in suspension culture.

2) Incorporation of ^{3}H and ^{14}C RNA precursors; scintillation counting.

3) Cell fractionation and RNA isolation.

4) RNA sedimentation in sucrose gradients.

5) Time-sharing computer.

6) Gel electrophoresis.

7) Electron microscopy.

Major Findings:

1) Effects of drugs on RNA metabolism in L1210 cells.

We had previously reported that BCNU and related compounds inhibit the maturation processing of nuclear 45s RNA to ribosomal RNA. This activity required the presence of the chloroethyl- and nitroso- groups, thus pointing to the N-alkyl-N-nitroso- part of the molecule as the active portion. It nevertheless seemed possible that the essential factor is the formation of isocyanates from the remainder of the molecule after cleavage and loss of the N-alkyl-N-nitroso- portion. Therefore, several isocyanate were tested. Chloroethyl isocyanate (NSC 87418), cyclohexylisocyanate (NSC 87419), and methylisocyanate (NSC 64323) were found to be active, thus substantiating the above possibility. Cyanate itself was inactive. Further studies showed that monochloroethylnitrosourea (NSC 47547) and chloroethylamine (NSC 10871) are inactive. The finding with cyclohexylisocyanate was followed up by experiments that showed that at a concentration of 10^{-4}M, incorporation of precursors into DNA and protein in L1210 cells are totally inhibited while incorporation into RNA is partially inhibited. The effects of this compound on other aspects of RNA synthesis, affected by BCNU, remain to be determined.

We had previously found that pulse-labeled RNA synthesized by L1210 cells in the presence of anthramycin, HN2, or camptothecin has a reduced chain length. This was understandable for anthramycin and HN2 which are known to bind irreversibly to DNA, but was puzzling for camptothecin which we had found does not bind to DNA. Studies on the effects of increased pulse duration have now shown that, although RNA chain lengths in the case of anthramycin and HN2 remain reduced, in the case of camptothecin the RNA chains increase towards normal lengths when the pulse is of sufficient duration. This indicates that camptothecin differs from the other two agents in that the effect on chain length of newly synthesized RNA diminishes at long incorporation times, as if it is due to a reduction in chain elongation rate.

The inhibition of RNA synthesis by camptothecin has been reported to be reversible. We have confirmed this finding in L1210 cells. In addition, we found that, after removal of camptothecin, the RNA synthesis patterns return to normal.

Progress on the study of drug effects on RNA synthesis in L1210 cells was impeded by the finding of contaminating Achyloplasma laidlawi in the cultures. The cultures were cured with kanamycin, and several uninfected clones were isolated. These uninfected cells, however, did not lend themselves to reproducible separation of nucleolar fractions, seemingly because of some structural difference in the nucleoli. The original infected culture was re-tested and found to fractionate properly. The infecting organism apparently does not contribute to the observed RNA labeling, as there was no detectable labeling of 16s or 23s RNA components in the cytoplasmic fractions.

Electron microscopy of our L1210 cell lines revealed the presence of intracisternal type A particles and budding type C particles. These were seen

in the original line, as well as in the kanamycin treated and cloned lines, and also in L1210 lines obtained from other laboratories. The content of virus particles was not reduced by passage through mice.

2) Measurement of drug-induced changes in chain lengths of RNA synthesized in L1210 cells.

Our previous studies of drug effects on RNA synthesis in L1210 cells had disclosed a reduction in the sedimentation of newly synthesized RNA, observed with certain agents known to bind tightly to DNA, but not in the case of agents that form rapidly reversible DNA complexes, (the comparisons being made for drug concentrations that produce equal extents of inhibition of RNA synthesis). Two unresolved problems impeded quantitative treatment of these data: (1) the sedimentation patterns showed considerable variability, especially near the bottom of the gradients, and were thought to reflect RNA aggregation; (2) it was not clear whether the RNA molecules sedimented as single chains or as aggregated structures. We have previously reported a partially successful means of circumventing this problem by means of a carefully graded reaction of the RNA with formaldehyde. We have now examined in more detail the effects of heat and formaldehyde on the sedimentation of nucleolar and nucleoplasmic RNA. We were particularly interested in examining for the presence of "hidden" single-strand breaks within double-stranded regions of RNA. We first looked for evidence of a thermal transition by heating the RNA to various temperatures prior to sedimentation. The results showed only progressive degradation of the RNA with time and with temperature. We then studied the effect of reaction with formaldehyde at 70°C, under conditions known to produce complete reaction with RNA. The results again showed a progressive degradation of the RNA. The data were found to fit a mathematical model for random degradation with an estimated rate of chain scission of 1.7×10^{-5} breaks per nucleotide per minute. There was no significant difference between nucleoplasmic and 45s nucleolar RNA. Similar studies at 40°C showed that 45s nucleolar RNA again fit the model for random degradation, the rate at 40° being estimated at 4.4×10^{-7} breaks per nucleotide per minute. Nucleoplasmic RNA at 40°, on the other hand, did not fit the random degradation model. The results were compatible with the idea that part of the nucleoplasmic RNA consists of "subunits" held together by moderately stable linkage, e.g. hidden breaks within double-stranded segments.

In the light of these findings, the effect of anthramycin on the sedimentation of nucleoplasmic RNA from treated cells was re-examined. Sedimentation was carried out after reaction of the RNA with formaldehyde at 40° or at 70°. The change in sedimentation pattern due to drug treatment was measured in mixtures of H^3-labeled RNA from treated cells and C^{14}-labeled RNA from control cells. The relation of H^3/C^{14} ratio to RNA molecular weight can be predicted from mathematical models for various mechanisms of RNA synthesis inhibition. The relation found at 40°, however, was such as not to fit any simple or likely model, whereas the data at 70° did fit models for random irreversible DNA-binding and premature termination of RNA chains. Since the data at 70° are complicated by random RNA degradation, the measurements were carried out after various formaldehyde reaction times. The results gave a consistent picture that leads us to believe that drug-induced changes in RNA chain legnths can be

quantitated despite a moderate degree of random degradation of the RNA. The results at 40° are probably influenced by the presence of hidden breaks, and so may not be suitable for quantitative testing of RNA inhibition models.

3) Ribonuclease-resistant RNA of L1210 cells.

Ribonuclease resistance has been used as an indicator of the helical conformation of RNA. Our interest in helical RNA in mammalian cells stems from two sources: (1) Our previous finding that intercalating agents such as proflavin, ethidium, and ellipticine, agents that would be expected to bind selectively to helical regions in RNA, inhibit the maturation cleavage of 45s nucleolar RNA ("NL-RNA") to form ribosomal RNA ("rRNA"). This suggested that NL-RNA might have helical segments at or near the points of maturation cleavage. (2) The finding by others that the RNA synthesized by actinomycin-treated cells has an increased ribonuclease resistance.

We had reported previously that ribonuclease resistance of uridine-labeled RNA of L1210 cells differs from that of viral double-stranded RNA in that the extent of ribonuclease resistance does not level off with increasing NaCl concentration. This indicates that helical RNA of mammalian cells consists of a heterogeneous class of short or poorly matched segments. We had also found that the kinetics of ribonuclease digestion of mammalian RNA are biphasic, indicating mixtures of sensitive and relatively resistant RNA segments. Our method of quantitating ribonuclease resistance is to extrapolate the slow component of the reaction back to zero time.

The significance of the biphasic kinetics and the importance of the extrapolation procedure have now been shown by an examination of poly A-poly [^{3}H]U, a homogeneous double-stranded molecule. This material gave mono-phasic kinetics, the rate constant decreasing with increasing NaCl concentration. This confirms that the ribonuclease resistance profiles of mammalian RNA measure the relative amounts of different types of RNA.

The possibility that the ribonuclease resistance at high NaCl concentration might be due to precipitation of the RNA was examined. It was found that above 1 M NaCl some of the RNA becomes sedimentable at 15,000 rpm, indicating aggregation or precipitation. This correlated with a flattening of the slow component of the ribonuclease digestion kinetics. Up to 1 M NaCl, however, there is no aggregation and the slow component of the kinetics maintain a constant slope. We are currently testing CsCl as a more suitable salt. Most of the results to be described, however, are based on NaCl concentrations up to 1 M. Cationic intercalating agents, including, proflavin, ethidium, quinacrine, and others, were also tested as potential helix stabilizers in ribonuclease resistance assays. Ribonuclease resistance did occur, as expected, at relatively low concentrations these agents; RNA aggregation, however, was a prominent feature, and this approach was not further pursued.

Comparisons between various RNA fractions from L1210 cells showed no significant difference in the ribonuclease-resistance profiles of NL-RNA and rRNA, but a somewhat greater degree of resistance in the case of nucleoplasmic RNA.

Thus, the extra helical segments of NL-RNA, if any, are too small or too few to be measured by this procedure. On the other hand, the results indicate a relatively high content of helical segments in nucleoplasmic RNA. The latter conclusion was confirmed using labeled cytidine ("C") instead of labeled uridine ("U"). The ribonuclease resistance of C was slightly greater than that of U in all cell fractions, as expected from the greater stability of helical segments containing G-C pairs relative to A-U pairs. The greater resistance of nucleoplasmic RNA was again seen indicating that it is not due to the difference in base composition.

The ribonuclease-resistant products were examined by gel electrophoresis to determine whether any high molecular weight species are preserved. All of the fragments were found to be comparable in size to tRNA, or smaller.

Several attempts to confirm the reported increase in ribonuclease resistance of RNA synthesized in the presence of actinomycin have been unsuccessful. The reason for the discrepancy is not established, but we think that our procedure more effectively removes non-RNA materials from the RNA preparations. We have also examined the possibility that intercalating agents may increase the amount of helical RNA in nucleoplasmic RNA or NL-RNA, with negative results.

Proposed Course:

1) Apply the technique that has been developed for measurement of size-distributions for measurement of size-distributions of newly synthesized RNA to other drugs.

2) Determine effects of drugs on nucleolar RNA processing in an *in vitro* system.

3) Proceed with studies of effects of camptothecin on RNA metabolism in L1210 cells.

4) Complete the current work on RNase-resistant RNA, after which this study will be terminated.

Publications:

1) Alan L. Snyder, Herbert E. Kann, Jr., and Kurt W. Kohn, J. Mol. Biol. (1971) 58: 555-565. Inhibition of the Processing of Ribosomal Precursor RNA by Intercalating Agents.

2) Herbert E. Kann, Jr., and Kurt W. Kohn, J. Cell. Physiol. (1972), in press. Effects of Anthramycin and Actinomycin on RNA Synthesis Patterns in L1210 Cells.

3) Herbert E. Kann, Jr., and Kurt W. Kohn, Molecular Pharmacol. (1972), in press. Effects of DNA-reactive Drugs on RNA Synthesis in L1210 Cells.

Serial No. NCI-6112
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1972

Project Title: Effects of Chemotherapeutic Agents on DNA

Previous Serial Number: Same

Principal Investigators: Kurt W. Kohn and Daniel Glaubiger

Other Investigators: None

Cooperating Units: Dr. Elliott Charney, NIAMD; (use of circular dichroism spectrophotometer and assistance with electric dichroism measurements).

Dr. David Ludlum, University of Maryland Medical School, Baltimore, Maryland; (use of light scattering photometer and assistance in making measurements).

Man Years

Total:	2.5
Professional:	1.5
Other:	1

Project Description:

Objectives:

To determine the mechanisms of interaction of chemotherapeutic drugs with DNA, and the consequences of such interactions on DNA structure and function.

Methods Employed:

1) Absorption spectroscopy; adaptations for kinetic measurements and thermal profiles.

2) Analytical ultracentrifugation.

3) Viscometry.

4) Light scattering.

5) Circular dichroism.

6) Electric dichroism.

7) Cell culture.

Major Findings:

A) Reaction of anthramycin with DNA.

1) Reaction Kinetics.

One possible way in which anthramycin could be used as a probe of DNA structure would be by utilizing the fact that the reaction rate is sensitive to DNA conformation, the rate being much faster with helical than with denatured DNA. In order to utilize this effect in quantitative studies, however, the reaction kinetics must be known. We had previously found that the reaction kinetics were not simple bimolecular, as had been hoped, and a consistent picture was elusive. Our previous studies had been done under conditions of DNA excess by measuring changes in the absorbance of anthramycin due to binding to DNA. We have now extended these studies to the region of anthramycin excess. To do this, a technique was worked out by which unreacted anthramycin could be rapidly and quantitatively extracted into an organic solvent. The only anthramycin remaining unextracted in the reaction mixture is that which has bound to DNA. These studies disclosed a consistent picture of anthramycin-DNA reaction kinetics as a bimolecular reaction with decreasing rate constant. The bimolecular rate constant decreases exponentially with extent of reaction, according to the relation,

$$k'(r) = k_o e^{-ar},$$

where r is the extent of reaction in molecules of anthramycin bound per nucleotide, k' is the apparent second-order rate constant, and k_o and a are constants which can be evaluated from the data. We believe that these findings are consistant with a neighborhood exclusion model, according to which a bound anthramycin interferes with further binding at neighboring sites. This fits with our previous observation that poly dG-poly dC reacts only to the extent of about 20% of its G's.

2) Nature of the Binding Site.

(a) Studies with Defined Polynucleotides.

We had previously found that reaction occurs exclusively with deoxypolymers containing GC. It was not clear however whether G or C or both is required. Single-stranded poly dG and poly dC were therefore tested separately. Reaction with poly dG, as with denatured DNA, was slow but clearly measurable. A small reaction with the poly dC sample was also found, but was accounted for by a 10% contamination of the sample with G. We therefore conclude that binding requires guanine.

(b) Competition with Actinomycin.

Since actinomycin also requires G for binding, a competition with anthramycin

for binding sites is anticipated. To test this, DNA was reacted to various extents with anthramycin and then titrated spectrophotometrically with actinomycin. A linear competition was observed between the binding capacity of DNA for the two antibiotics.

(c) Circular Dichroism.

Anthramycin at neutral pH was found to have a biphasic pattern. This changes to a broad monophasic pattern above 8.5, where the phenolic OH ionizes. The anthramycin-DNA complex at neutral pH resembled that of free anthramycin above pH 8.5. We conclude that in the complex, the phenolic OH is either hydrogen-bounded or ionized (the latter is unlikely on structural grounds). This conclusion is supported by our previous finding by acid-base titration that the dissociation at pH 8.5 of anthramycin is suppressed in the complex.

(d) Physical Studies.

We had previously observed a marked increase in DNA viscosity upon reaction with anthramycin. This appeared to be due neither to intercalation nor to aggregation. In order to clarify the origin of this effect, sedimentation and viscosity were measured for complexes made with DNA preparation of different molecular weights, ranging from 10^5 to 10^7. The viscosity increase almost disappeared as the molecular weight of the DNA was reduced. Such behavior would tend to implicate aggregation as a cause of the viscosity increase, but this possibility was excluded by the absence of significant changes in sedimentation constant. We interpret these findings to indicate that anthramycin stiffens the DNA helix without producing an increase in length. Thus, the DNA molecules of the low molecular weight preparations were short enough to be essentially straight rods; therefore there could be no further stiffening and hence no rise in viscosity.

This interpretation was further tested by light scattering measurements. Anthramycin was found to increase the radius of gyration of DNA with no change in molecular weight. The percentage increase in radius of gyration was about 1/3 of the percent increase in viscosity, as expected from theory. In the low molecular weight preparations, the change in radius of gyration was greatly reduced, showing that molecules that were short enough to be straight were not lengthened. The increase in radius of gyration in the higher molecular weight preparations must therefore be due to stiffening of the helix.

The major conclusion is that when anthramycin binds to helical DNA, the flexibility of the helix is reduced. The DNA molecule is not lengthened, contrary to the intercalation mechanism.

Measurements of electric dichroism were initiated, in collaboration with Dr. Elliott Charney, NIAMD. Early results have shown that the plane of the anthramycin ring system is approximately perpendicular to the plane of the DNA bases.

2) Summary

We have developed the following picture of the anthramycin-DNA complex: Anthramycin is bound through a labile covalent bound requiring the presence of guanine and the absence of 2'-hydroxyl groups. The phenolic OH of anthramycin is linked to the DNA, possibly by hydrogen bonding. The plane of the anthramycin chromophore is oriented perpendicular to the DNA bases, and the molecule is placed so as to brace the DNA helix and inhibit its bending.

B) Effects of anti-tumor agents on the intracellular state of DNA.

Studies were initiated of the possibility that aspects of the intracellular state of DNA could be measured by filtration techniques. The aspects of interest are (1) DNA attachment to other structural components of the nucleus and (2) DNA strand breaks. The principles involved are (1) DNA fragments might be retained on a filter only if attached to another component that either is too large to pass thru the filter or that adsorbs to the filter material, and (2) dissociation of DNA fragments from filter-trapped material may be facilitated by the presence of strand breaks. The following results encourage us to believe that these principles can be put into practice: (1) DNA from cell lysates can be quantitatively retained on filters under conditions in which most of the other cell material passes through; (2) shearing of the lysates prior to filtration reduces the fraction of the DNA caught by the filter; (3) under suitable conditions, the filter retention of DNA fragments from sheared cell lysates is several times greater for newly synthesized DNA than for over-all cell DNA; (4) over-all cell DNA quantitatively trapped on a filter is not released by denaturing solvents, but a part of the newly synthesized DNA is released; (5) x-irradiation of the cells allows the filter-trapped DNA to be released by denaturing solvents.

Significance:

Detailed study of the anthramycin-DNA interaction is of special interest for two major reasons. (1) The drug constitutes a unique chemical class of DNA-binders, and has an unusually high degree of affinity and specificity for DNA. The chemical mechanism of binding is unknown and is not related to that of any previously studied compound. The elucidation of this mechanism could permit the design of other chemical structures that would retain the DNA reactivity but perhaps not all of the toxicity. The molecule lends itself to this approach because of its relatively small size. (2) The high degree of specificity for helical DNA and the near irreversibility of the reaction appear to place anthramycin in a class by itself. Because of these properties, the molecule is potentially useful as a probe of DNA conformation in complex structures.

Proposed Course:

(1) With the completion of the electric dichroism measurements, the anthramycin study described above will be complete and ready for publication.

(2) Further studies are planned with two-stranded oligonucleotides of known sequence in an attempt to prepare a defined complex with anthramycin that may be suitable for detailed structural analysis, perhaps by X-ray crystallography.

(3) If the outcome of methodologic studies continues to be favorable, work will be concentrated on the effects of anti-tumor agents on the intracellular state of DNA.

Publications: None

SUMMARY REPORT

LABORATORY OF TOXICOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 - June 30, 1972

Efforts during the past year to improve the Chemotherapy Program-support role (preclinical toxicology) have resulted in an increased efficiency and responsiveness in regard to this aspect of the Laboratory of Toxicology's responsibilities. In addition, each senior investigator has continued to develop his independent basic research effort, and this progress has been very encouraging. These programs will be mentioned in more detail later in this report. The Laboratory of Toxicology is attempting to blend program-support activities with solid basic research having application to cancer therapy. The current standard procedures for preclinical toxicologic evaluation of cancer chemotherapy agents has just been completed and copies sent to our present contractors. This protocol reflects the experience gained during the conduct of preclinical toxicology over the past years. The Second Chemotherapy Conjoint Meeting at Annapolis provided an opportunity to discuss and resolve most of the past problems associated with the general protocol. Each of the individual contractors, who actually carries out the toxicologic studies, made valuable suggestions.

A more recent undertaking has been the formulation of guidelines for the pathologic evaluation of tissues in regard to preclinical toxicology. This new guideline is entitled "Protocol for the Pathologic Evaluation of Dogs and Monkeys Utilized for Preclinical Toxicologic Testing of Antineoplastic Agents." Codes were developed to identify organ systems, morphology, localization, semiquantitative ratings, and definitions of lesions in an effort to facilitate record keeping and adapt these pathologic data for card-punching at the present time and computerization in the near future. This has been a major undertaking, and will result in more reliable preclinical pathology to support the introduction of a new anticancer drug into the clinic.

A major problem in the prediction from preclinical toxicology continues to be a failure to predict some of the more unusual drug-induced toxicities. For example, platinum has produced audiotoxicity and deafness in patients following prolonged treatment with high doses. Unfortunately, analysis of the large animal toxicology did not indicate this drug hazard. Obviously, better testing procedures are needed for drug toxicity associated with the special senses such as hearing and sight. Central nervous system and cardio-vascular toxicity also present special problems. Efforts are being undertaken to develop and employ better test procedures in all of these areas. Additional efforts and emphasis are also being placed on the long-term

toxicity of antineoplastic agents, and the effect of anticancer drugs on reproduction. Future consideration is required to carefully assess the mutagenic potential of anticancer drugs which are being used in an ever-increasing number of non-neoplastic diseases.

A unique resource of the Laboratory of Toxicology is the extensive accumulation of toxicologic studies available for retrospective analysis of predictive ability when coupled with the carefully documented clinical trials. A continuing effort is required to define the qualitative and quantitative predictiveness of large animal toxicology for the clinical situation. These retrospective studies will retain a high priority in the coming year.

The independent projects of each senior staff member continues to contribute to the basic understanding of drug-induced toxicity. It is apparent that more information is needed to determine and understand the mechansism of drug-induced disease. This knowledge will hopefully allow for the future control of toxicity with a resulting increase in clinical efficacy of new and currently used antineoplastic agents.

One group in the Laboratory of Toxicology has continued to work on selected problems of a biochemical nature. These problems include a continuing systematic search for powerful irreversible inhibitors of L-asparagine synthetase which might be valuable therapeutically to prevent or delay clinical resistance to L-asparaginase. Surveys are being conducted on the distribution of L-asparagine synthetase in the organs of a variety of mammals, plants, and helminths. To date, it has been established that, in mammals at least, the exocrine pancreas is the richest site of L-asparaginas biosynthesis. Studies of L-asparagine covalently bonded to dacron graft material are also continuing. The Michaelis constants of the bonded enzyme are higher by approximately two logs than those of the free enzyme, the V_{max} of the bonded enzyme is relatively more insensitive to large shifts of pH than the free enzyme, and the bonded enzyme is altered by exposure to the substrate analogue, DONV as indicated by a lower K_m. During the past year streptozotocin toxicity has been studied by this group. In order to determine whether streptozotocin depresses NAD in the islets of Langerhans (as it does in the liver) a convenient radiometric technique for measuring as little as 100 femtomoles (1×10^{-13} Moles) has been developed. It has been found that streptozotocin does depress insular NAD and that nicotinamide prevents the depression. Preliminary studies of mice treated with very large single doses of streptozotocin have revealed that guanyl residues of DNA are being alkylated in the 7 position. This finding may shed light on the mechanism of the known carcinogenicity of streptozotocin.

The Laboratory of Toxicology has expanded a major effort in the area of data analysis where we continue to evaluate the quantitative relationships between the toxicity of anticancer agents in preclinical species (dogs and monkeys) and man. Publication of this work in complete form during the coming year will allow for a more rational extrapolation of the initial Phase I clinical dose from preclinical toxicologic studies.

The comparative pathology group has played an active role in the efforts to improve the pathology which is done in support of the general preclinical toxicology. Members in this group are involved with studies to detect, quantitate, evaluate, and predict the effects of antineoplastic agents on the hematic system. Most of the anticancer agents have adverse effects upon the bone marrow of treated patients and it is well known that such treated patients are quite susceptible to bacterial and fungal infections. In fact, some of the patients who are cured of cancer can get into serious difficulties from infections to which they are susceptible because of the anticancer agent. It takes approximately 5 days before toxicity to the bone marrow is expressed by a decrease in the numbers of circulating white blood cells. Methods to detect toxicity based upon the number of cells in the bone marrow reserve are under development in order that the bone marrow toxicity might be recognized sooner and appropriate measures can be taken to protect the patients.

Others in this group have also been very active in the Chemotherapy Program-support area by providing guidelines to standardize, semiquantitate, and computerize the pathologic evaluation of experimental animals utilized by contractors in preclinical toxicologic testing of new anticancer drugs. Retrospective analysis of experimental animal and human data have been done to evaluate and record reports of pathologic effects of antineoplastic agents in man and correlate these findings with those recorded in test animals. In this effort more than 200 cancer patients have been studied retrospectively. Additional studies of the mechanisms of antineoplastic drug-induced pathologic alterations include a consideration of the daunomycin and adriamycin induced cardiomyopathy, and the pathogenesis of L-asparaginase-induced hypocalcemic tetany in rabbits. Studies of the mechanism of hemolysis induced by ellipticine are also being undertaken in an attempt to resolve this problem.

The effect of antineoplastic agents on various aspects of reproduction are of interest to the Laboratory of Toxicology. Some members have continued to study the biochemical and functional effects of anticancer agents on spermatogenesis. These studies utilize the velocity sedimentation cell separation technique and *in vivo* serial mating. Attempts are made to correlate the biochemical effects of the test drug on thymidine, uridine, and L-leucine incorporation by selected spermatogenic cell types with the decreased fertility as revealed by serial mating. Many of the clinically useful anticancer drugs have been studied in an attempt to anticipate and predict clinical toxicity where spermatogenic effects have not been recorded, and to define those drug-associated reproductive effects that are already known, e.g. procarbazine. Also in this section some are evaluating the effects of prenatal exposure to antineoplastic agents on the subsequent reproductive capacity of the offspring. These studies include the investigation of the mechanisms involved in the production of subfertility in adult animals as a result of their *in utero* exposure to anticancer agents, and an assessment of the transplacental carcinogenic potential of various antineoplastic drugs. These studies are especially important because the embryonic development of the mammalian gonads is unique in that all the DNA synthesis takes place within a period of a few days. In the male no further DNA synthesis then takes place until puberty, and in the female no further

oocyte DNA synthesis takes place during the lifetime of the animal. Because of clinical reports regarding the effects of procarbazine and cyclophosphamide on reproduction, these two drugs are being evaluated first. The interest in the *in utero* carcinogenic potential of anticancer agents was prompted by the many recent clinical reports of vaginal cancer in young women associated with their prenatal exposure to diethylstilbesterol.

In another important section of the Laboratory, large volumes of toxicology information are handled. This input is generated largely from our contractors employing the standard protocol developed by us. The reports must be summarized and distributed to persons and agencies needing information on toxic doses and toxic effects of antineoplastic agents.

Serial No. NCI-3507
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Preclinical Toxicology of Drugs Potentially Useful in Cancer Chemotherapy

Previous Serial Number: Same

Principal Investigator: Elton R. Homan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Others:	0

Objectives:

The objectives of this project are 1) to evaluate the toxicologic and pharmacologic properties of potential cancer chemotherapeutic agents in animals for the purpose of expediting clinical trial, 2) to develop better predictive models for the study of toxic effects of chemotherapeutic agents. 3) to study the influence of drug toxicity of dosage scheduling, concomitant drug administration, and alteration of environment variables, and 4) to facilitate the handling and use of toxicologic data generated by the laboratory and its contractors by development of data processing methods.

Principal Findings:

Relationships between toxic levels of antitumor drugs in experimental animals and man.

Investigations previously undertaken related the maximum tolerated dose (MTD) in animals to that in man. It has become apparent that the term MTD must be loosely interpreted to encompass the usages of the term by various investigators. In an attempt to refine the quantitative description of dosage relationships, the Laboratory of Toxicology promulgated definitions of the highest non-toxic dose (HNTD), toxic dose-low (TDL), toxic dose-high (TDH), and lethal dose (LD).

A study is being made of the quantitative relationships among the above dosage levels observed in experimental animals and between these levels and clinical doses. The initial phase was a review of toxicity data of antitumor compounds for which IND's have been filed. To date, over three hundred dosage regimens of drugs administered to dogs and monkeys have been examined and the available defined dosage levels tabulated. Analyses of these data are under way.

Another aspect of this investigation involves examination of the cumulative toxicity associated with repeated dosing. One of the simplifying assumptions made in the earlier work was that the effective toxic dose (ED) was simply the product of unit dose (UD) and number of doses (N) given,

$$ED = UD * N,$$

irrespective of the dosage frequency or schedule. It is evident from the current work that this model leads to gross errors. It is possible to assume, however, that the expression represents a special case of a more general relationship,

$$ED = UD * N^k,$$

where, in the special case, k = 1. The value k represents the tendency of responses of an animal species to accumulate during a specified dosage regimen. Values of k less than unity indicate relative safety of prolonged administration of a given regimen, values greater than one, increasing toxic risk from continued administration. The logarithmic expression of the above equation,

$$\ln(ED) = \ln(UD) + k \ln(N),$$

is linear and suggests a simple way of estimating the ED for any value of N.

Values of k have been calculated for about fifty cases, including the several toxic levels and various regimens. Observed values range from k = 0, implying complete recovery between sequential doses, to about k = 2, corresponding to a marked accumulation of toxic effects with repeated dosing. The k's for various regimens of each drug tend to be clustered, but there are patterns related to the regimens and toxic levels as well.

Comparative examination indicates that cautious prolongation of therapy is especially important in the use of drug regimens associated with high k values. In these cases, the k values for lethal doses tend to be higher than the k's for minimally toxic doses (TDL or TDH, depending on the slope of the dose-response curve). This implies that the reversibility of toxic effects diminishes markedly in these cases as therapy is continued.

Statistical Analysis of Clinical Chemical and Hematologic Data in Experimental Animals - A Feasibility Study

Valid interpretation of clinical chemical and hematologic observations in toxicologic studies is fundamental to the determination of useful guidelines

for clinical introduction of new agents. The sheer bulk of this data in any study has rendered its full utilization difficult or impossible. In addition, interpretation is somewhat subjective and the significance of patterns of alterations may very well be over- or understated or even missed entirely.

Mason Research Institute has published a semiquantitative toxicity rating system which is of considerable benefit because the expression of changes in the various parameters are a consistent numeric notation. Without detracting from its utility, the method is nonetheless judgemental and arbitrary in the definition of ratings. Furthermore, extension of the system to other laboratories would require validation of the ratings in each laboratory. On the basis of these considerations, a search was undertaken for a means of unifying clinical chemical and hematologic data which would be consistent among laboratories and based on observation rather than judgemental criteria. The method which evolved is based on changing the question implicit in examining the data: "Is this value abnormal?" becomes "How probable is this value?" Since an observation has some probability of occurrence in a given context, the numeric value of that probability, or some function of it, could be the basis of unification.

The common approximation of the logarithmic Gaussian distribution to biologic phenomena makes it a logical candidate as a means of data reduction. The distributions of pretreatment values of between thirty and thirty-five chemical and hematologic parameters, in each of several preclinical drug studies, were analyzed. The procedure used was the conversion of rank values of the ordered array of observations to standard deviational units (SDU) using a rational approximation and regression analysis of SDU on log observations. The linear fit was quite good for most parameters.

Significance to Bio-Medical Research and the Program of the Institute

The limitation imposed on cancer chemotherapy by clinical toxicity requires a continuing search for appropriate experimental animal models. Two complementary goals are involved in this search: 1) improved systems for predicting toxicity and 2) means of circumventing or ameliorating toxic effects. Implicit in both of these is the need for an understanding of pertinent physiological mechanisms which permits rational extrapolation.

Thus, the value of the studies carried out under this project to the larger program is twofold. In addition to providing toxicologic data on specific antitumor agents of present or potential clinical interest, the studies are intended to develop tools for subsequent investigation which are more sensitive or predictive.

With regard to data processing activities, there is increasing awareness in the toxicology community at large, as well as in the Laboratory of Toxicology, of the need for more effective ways of handling and presenting toxicologic data. The work being performed is intended to develop the capabilities of the laboratory in the area of data processing and to determine the extent to which contractors can participate effectively in these activities.

Proposed Course

1. Relationship between toxic levels of antitumor drugs in animals and man.

   The methodology developed during the current year opens the way for a more realistic appraisal of the relationships between animal and clinical toxicology. In cooperation with the Cancer Therapy Evaluation Branch, the examination of current alternative quantitative models of dosage prediction (mg/M^2 vs. mg/kg) will be continued. It is hoped that a definitive resolution of the question of the "best" model can be achieved. It may be possible to determine whether each of the models is applicable to particular drug classes.

2. Biliary excretion of organic acids.

   Hart et al. have reported that the organic acid, phenolsulfophthalein (PSP), commonly used in the evaluation of renal function, is actively secreted into the bile in significant amounts, in part as the glucuronide. The present investigation is an extension of this work. Sequential bile samples from PSP treated rats were analyzed colorimetrically for total and free PSP. Glucuronide conjugation was demonstrated by cleavage with glucuronidase and thin layer chromatograpy in two different solvent systems. PSP in both bile and plasma have been examined. The study has thus far confirmed the conclusions of Hart and, in the future, will include examination of renal and hepatic excretion of phenolsulfophthalein in rats. Comparative examination of this phenomenon in Wistar and Gunn rats will be carried out with measurement of free and conjugated dye in bile, plasma, urine, kidney and liver. On the basis of methodology in this work, it should then be possible to extend the approach to other organic acids including appropriate antitumor drugs.

3. Statistical analysis of clinical chemistry and hematology.

   The conversion of data from printed tables to computer files and proofing of these data for the studies outlined earlier have proved very time consuming. The analyses undertaken will be completed, but no significant enlargement is anticipated and the work will not be given a high priority. While the potential rewards from this work are significant, the application of the methods to animal studies does not appear to be practicable at the present time.

Publications: Serial No. NCI-3507

1. Homan, E. R. Quantitative relationships between toxic doses of anti-tumor chemotherapeutic agents in animals and Humans. Cancer Chemo. Reports, part 3, vol. 3, #1, pp 13-19, 1972.

2. Homan, E. R., Zendzian, R. P., Schott, L. D., Levy, H. B., and Adamson, R. H., Studies on Poly I:C toxicity in experimental animals., Tox. Appl. Pharm., 1972 (In press).

3. Primack, A., Young, D. M., Homan, E. R., Multiple congenital anomalies in a Rhesus monkey: A case report. Teratology, Vol 5, April 1972, 137-141.

Serial No. NCI-3518
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: L-Asparagine: Synthesis, Biological Role and Hydrolysis

Previous Serial Number: Same

Principal Investigator: David A. Cooney

Other Investigators: Hiremagalur Jayaram
Ruth D. Davis
Harry Milman

Cooperating Units: Howard Weetall, Lee Hersch, Corning Glass Company, Corning, New York
John Driscoll, Chemotherapy, National Cancer Institute

Man Years:

Total:	2 1/2
Professional:	1 1/2
Others:	1

Objectives:

It is the objective of the present research to characterize L-asparagine synthetase from neoplastic and normal tissue - and to determine the most efficient means of inactivating that enzyme. It is hoped that an active chemotherapeutic agent will result from such an approach.

Principal Findings:

Continuing Studies of L-Asparaginase

Studies of L-Asparagine Synthetase.

During the past fiscal year numerous compounds have been tested as potential inhibitors of L-asparagine synthetase, utilizing both ammonia and L-glutamine as amidodonors.

Name	Concentration	Serial No. NCI-3518 % Inhibition with L-Glutamine as substrate	Serial No. NCI-3518 % Inhibition with NH_4^+ as Substrate
Urea	1×10^{-3}M	0	0
Urea	1×10^{-2}M	40	82
Urea	1×10^{-1}M	72	74
Neocarzinostatin	1×10^{-3}M	9	6
Thiazolidine-4-carbolic acid	1×10^{-3}M	15	8
3-Bromopropionic acid	1×10^{-3}M	0	9
Hydroxy-urea	1×10^{-3}M	0	9
Indole-2-carboxylic acid	1×10^{-3}M	0	10
Acrylamide	1×10^{-3}M	20	11
Methylene-L-glutamine	1×10^{-3}M	8	14
Hydrazine hydrochloride	1×10^{-3}M	16	15
Diamide	1×10^{-3}M	89	87
Diamide	1×10^{-4}M	30	50
Diamide	1×10^{-5}M	0	18
Triethylamine borane	1×10^{-3}M	17	22
Pyromellitic dianhydride	1×10^{-3}M	19	24
Streptovitacin A	1×10^{-3}M	25	25
2,6-Piperazinedione, 4,4'-ethylenedi-	1×10^{-3}M	0	25
2,6-Piperazinedione, 4,4'-propylenedi-	1×10^{-3}M	0	25
β-methyl-DL-aspartic acid	1×10^{-3}M	25	25
6-Amino nicotinamide	1×10^{-3}M	18	27
5-methoxy,4-bromo-2-oxo-dihydrofuran	1.5×10^{-3}M	47	89
5-methoxy,4-bromo-2-oxo-dihydrofuran	1.5×10^{-4}M	0	27
Trimethylamide borane	1×10^{-3}M	0	28
2,4-thiazolidinedione	1×10^{-3}M	0	29
2-Amino-3-phosphopropionic acid	1×10^{-3}M	33	31
Mucobromic acid	1×10^{-3}M	27	37
Cis-diammino-dichloro-platinum	1×10^{-3}M	28	39
5-methoxy,3-bromo-2-oxo-dihydrofuran	1×10^{-3}M	23	42
5-methoxy,3-chloro-2-oxo-dihydrofuran	1×10^{-3}M	0	43
2-Amino-4-phosphobutyric acid	1×10^{-3}M	37	46
Hydroxylamine	1×10^{-3}M	0	61
5-methoxy-4-chloro-2-oxo-dihydrofuran	1×10^{-3}M	44	67
Mucochloric acid	1×10^{-3}M	32	84

The following drugs were tested as potential inhibitors of L-asparagine synthetase, however, no inhibition was found:

Succinonitrile
5-FU
Tritylcysteine
5-methoxy-2-oxodihydrofuran
Phosphonacetic acid
5-chloro-2-oxodihydrofuran
5-Hydroxy-2-oxodihydrofuran
Pyrrole-2-carboxylic acid
2,3-Pyrazine dicarboxylic acid
2,3-Pyridine dicarboxylic anhydride
Oxamic acid
1-Methylhydantoin
Pyromellitic diamide
2,3-Pyridine dicarboxylic acid
Glutarimide, 3-[2-(5-acetoxy-
3,5-dimethyl-2-oxocyclohexyl)-
2-hydroxy-ethyl)
Methyl L-glutamate
DL-penicillamine
Crotonamide
Fluoracetate
Diglycolic acid
Carboxymethoxylamine hemihydrochloride
2-Pyrazine carboxylic acid
6-Azauracil
Succinic acid dihydrazide
Phloroglucin
L-Ascorbic acid
L-2-azetidine carboxylic acid
Diaminomaleonitrile
Methylene diphosphonic acid
β-γ-Methylene adenosine 3 phosphate
Thiocarzolamide
Carzolamide
D-biotin
Streptozotocin cofactor, Zedalan
Actadione, thiosemicarbazone
Pyrazomycin
γ-Glutamyl methylamide

It is of considerable interest that the halogenated organic acids, mucochlorate and mucobromate, are effective inhibitors of the enzymatic synthesis of L-asparagine only when ammonium ion is utilized as the source of the amide. Pursuant to this finding a large number of halogenated cyclic organic acids were requested from and sent to the NCI by Dr. F. Farina of the Instituto de Quimica Organica General, Madrid, Spain. Several of these (notably - 5 methoxy-4-bromo-2-oxo-dihydrofuran) were powerful inhibitors of L-asparagine synthetase; but none exceeded mucochloric acid in potency. Since L-asparagine synthetase is a 'sulfhydryl-enzyme', (subject to inactivation by PCMB, NEM, Ethacrynic acid and Diamide) it is not impossible that these halogenated organic acids are inhibitory by virtue of sulfhydryl blockade. Studies are therefore underway to determine whether 'thiol protectors', such as Celands's reagent, thiodiglycol and mercaptoethanol will antagonize the subject inhibition.

The inhibition of L-asparagine synthetase by urea is also worthy of comment. At 1 x 10^{-2}M, urea inhibits the enzyme $\sim$ 80%. This concentration of urea is unlikely to influence the secondary or tertiary structure of the enzyme. Now, in most mammals with normal renal function the BUN approximates 20 mg %, i.e. $\sim$ 3 x 10^{-3}M; in most inhibition studies with the crude enzyme, this concentration of urea is only marginally inhibitory. But in uremia, when the BUN may range from 30 - 300 mg %, it is not unlikely that L-asparagine biosynthesis will be profoundly deranged. Experiments are, therefore, projected to study the removal-rate of exogenous ^{14}C-L-asparagine in dogs rendered uremic with diamminodichloroplatinum.

A new technique for estimating the total-body-biosynthetic rate for L-asparagine will also be applied to these animals.

L-Asparagine Synthetase in Pancreas

Only a few surveys of the distribution of L-asparagine synthetase in the organs of mammals have been carried out and, though few, they have restricted themselves to the assay of a relatively small number of organs. Holcenberg for example studied the guinea pig and reported that washed intestine exhibited the highest activity. In this laboratory the isolation of a normal L-asparagine synthetase - as opposed to the better-studied tumoral enzyme - was felt to be worthwhile on several scores: the two enzymes could and should be compared, kinetically and immunologically; and, the relative inhibition of the normal vis-a-vis the tumoral enzyme by the various inhibitors uncovered to date is of some importance from a toxicologic point of view. However, we were unable to confirm Holcenberg's finding of consistent abundant intestinal L-asparagine synthetase. It was observed however that many (but not all) of our intestinal extracts (high-speed supernatants were used) exhibited considerable synthetase activity. An examination of the surgical technique used to excise the intestine revealed that small fragments of pancreas strongly resembling serosal fat or appendices epiploicae, were closely adherent to the bowel along the proximal duodenum. So then the whole murine

pancreas and later isolated islets therefrom were examined for L-asparagine synthetase. An abundant activity was detected only in exocrine pancreas. The pancreas from ten other vertebrate species have now been examined and it has uniformly been found that the pancreas is the richest site of L-asparagine biosynthesis.

Studies with Azotomycin

Last year it was reported that single high doses of Azotomycin (Glutamine, N-[1-[(1-carboxy-5-diazo-4-oxopentyl)carbamoyl]-5-diazo-4-oxopentyl]-, sodium salt) the Pfizer diazo-antibiotic, produced a striking elevation of hepatic L-glutamine. During the present fiscal year, studies of the mechanism of action of Azotomycin have continued. It has been established that Azotomycin, and DON, its close congener, are causative of convulsions and spasticity in mice when the drugs are given at doses $\geq$ 600 mg/kg. Attempts to moderate this seizure activity with L-glutamine, L-asparagine, adenine HCl and L-asparaginase have met with failure.

Measurements of CNS levels of L-asparagine in mice convulsing as an consequence of Azotomycin treatment were undertaken in the light of the observation of Canellos, and of ourselves, that the drug is a powerful inhibitor of L-asparagine synthetase. Indeed, clinical combination therapy of ALL, using Azotomycin and L-asparaginase has been undertaken - with largely negative results - on the basis of this inhibition. L-Asparagine in the cerebrum of convulsing mice was found to be significantly lower than in control subjects. Measurements of L-asparagine synthetase in the brains of intoxicated mice have shown that Azotomycin - somewhat paradoxically - passes the blood brain barrier and inhibits cerebral L-asparagine synthetase > 50% after a dose of 600 mg/kg. The duration of this inhibition remains to be determined. It is also paradoxical that Azotomycin which historically at least has been categorized as an antagonist of L-glutamine, failed to alter cerebral levels of that amide and wholly failed to inhibit cerebral L-glutamine synthetase, in vivo.

Studies of L-Asparaginase Covalently Bonded to Dacron Prostheses and to Glass Plates.

During the past year, biochemical studies of bonded L-asparaginase have continued. The Michaelis constants, reported by Handschumacher and Jackson of free L-asparaginase from E. coli for its four principal substrates are lower, by at least 1 log, than the same constants of L-asparaginase bonded to a Dacron vascular prostheses. The coupled enzyme has become a 'low affinity' L-asparaginase - a feature considered by Broome and others to be an undesirable feature in an enzyme intended for chemotherapeutic use. It is interesting that different graft-preparations (prepared by identical techniques) exhibit somewhat different Michaelis constants. It has also been found that the covalently-bonded L-asparaginase is susceptible to inactivation by DONV in a manner qualitatively similar to the free enzyme.

Some residual activity (∿ 3%) persists after exposure to DONV, in both cases. However, whereas the Km of the free residually-active enzyme is the same as that of native, unexposed (to DONV) enzyme, the Km of the bonded enzyme has fallen by one or two logs. Thus, DONV is likely to have alkylated and blocked most of the active sites on L-asparaginase but also to have alkylated some other site; and this latter alkylation has improved the catalytic properties of the residual active sites.

Systematic studies are also underway with bonded and free L-asparaginase to determine the comparative influence of pH and temperature on the affinity constants of this enzyme. To date it has been found that the Km of the free enzyme rises by > 1 log as the pH falls below 4.6, whereas the Km of the bonded enzyme is approximately the same at pH 4.6 as it is at pH 8.4, the pH optimum of free E. coli L-asparaginase. This finding is interpreted as follows - in the case of the free enzyme, at pH 4.6 ionizable groups, at or affecting the active site, dissociate and impair the entry of L-asparagine; in the bound enzyme these sites are either involved in the covalent dacron-to-protein linkage, or else that linkage has so restricted the secondary structure of the hydrolase that conformational perturbations brought about by pH cannot take place.

L-Asparaginase has also been covalently bonded to glass plates. The plates have been then incorporated into a hydraulically apt extracorporeal perfusion apparatus to the inlet of which, after minor surgery, an arterial connection can be made. The effluent is returned to the donor via a great vein. To date extracorporeal perfusion of 6 dogs and 2 baboons have been carried out with this device. It has been found that systemic plasmatic L-asparagine could be markedly depressed or eliminated for periods of up to three hours by this device. It has also been observed that there is a small leakage or leeching (< .01%) of active L-asparaginase from these plates. Since the grafts and plates are being utilized as a "non-immunogenic" means of depressing plasmatic L-asparagine, this finding is of some importance. To date, however, no antibodies to EC2 have been detected in the serum of animals which have undergone extracorporeal perfusion.

In order to measure the leeching-off of L-asparaginase, it was necessary to develop a technique capable of detecting ∿ 5×10^{-6} IU/ml (of perfusate) of L-asparaginase. A radiometric technique with this sensitivity has been developed. UL $^{-14}$-C-L-asparagine, 1 μCi, is added to the sample of raw plasma. After 4 hours at 37°C the production of L-aspartic acid is quantitated by the zinc decarboxylation technique described last year. One interesting by-product has emerged from the utilization of this technique: the plasma of some representatives of many species does contain approximately 1×10^{-5} IU/ml of L-asparaginase. Whether this amidohydrolytic activity represents autochthonous (hepatic) enzyme or bacterial enzyme remains to be determined.

Purification of L-Asparaginase from Dasyprocta Aguti

Over the past several years the National Cancer Institute has procured approximately 10 liters of agouti plasma, by cardiac puncture. The L-asparaginase from this species is devoid of L-glutaminase. Attempts are underway to purify the enzyme so that it can be used for murine-antitumor testing and toxicology in a small number of Rhesus monkeys and rabbits, species unusually susceptible to the toxicity of L-asparaginase. To date only partial success has been achieved because the enzyme becomes progressively more unstable with purification. This is in confirmation of the preliminary studies of Meister done under contract to NCI last year. The following steps are being used: 1) a 35% NH_2SO_4 cut brings down greater than 90% of the agouti L-asparaginase with a 12 fold increase in specific activity, 2) after passage through Sephedex G-25 to remove NH_4^+ the enzyme is absorbed batch wise onto DEAE-cellulose. Depending on the amount of absorbant used, L-asparaginase is eluted with 0.2 or 0.5 M phosphate with a ten fold increase in specific activity, and a 70% yield. The next step has yet to be decided on: preparative acrylamide gel electrophoresis, absorption to asparaginyl agarose and Sephedex G-200 chromatography will all bring about substantial purifications but there are technical drawbacks to each of these steps and the enzyme becomes labile with their employment.

Studies with Streptozotocin

Collaborative studies on the mechanism of action of the diabetogenic antibiotic streptozotocin (NSC-85998) [glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-], are described in full elsewhere (cf P.S. Schein, NCI) In the present report only those studies originating in the Laboratory of Toxicology will be described.

Measurement of NAD in the Isolated Islets of Langerhans

Streptozotocin has been shown by P.S. Schein to depress markedly the concentration of NAD in liver of the intoxicated mice. It has suggested that--were this same depression to be operative in the islets of Langerhans glucose and energy metabolism might be radically deranged. Such a sequence of events could be envisioned as contributing to β-cell death. We, therefore, resolved to measure the influence of streptozotocin, given intravenously at diabetogenic doses, on the concentration of pyridine nucleotides in isolated islets of Langerhans.

In view, however, of the small tissue size involved (islets weigh from 1 to 10 μgrams), high sensitivity (to < 0.2 picomoles) was needed for estimating the levels of NAD. Enzymatic fluorometric cycling techniques in our hands were plagued by prohibitive "blank-values". We, therefore, attempted to develop a radiometric cycling assay for NAD.

Utilizing 1-^{3}H-ethanol of high specific activity, cold, pure pyruvic acid, and charcoal-treated (NAD-freed) lactate dehydrogenase (LDH) and alcohol

dehydrogenase from yeast (ADH) the following cycle was caused to operate in the presence of NAD.

$$NAD + {}^{3}H\text{-Ethanol} \xrightarrow{ADH} \text{Acetaldehyde} + {}^{3}H\text{-NADH}$$

$$^{3}H\text{-NADH} + \text{Pyruvate} \xrightarrow{LDH} {}^{3}H \text{ Lactate} + NAD$$

NAD can be seen to operate in a catalytic way. At the termination of the incubation period, the unreacted ^{3}H-ethanol and ^{3}H-acetaldehyde are simply evaporated at 95°C in a hood. The residual ^{3}H-lactate is dissolved and counted in a scintillation spectrometer. About 0.1 picomole of NAD can be measured this way.

With the development of this radiometric technique, it was possible to determine the influence of streptozotocin on the levels of NAD in mouse islets. A single intravenous dose of 200 mg/kg depressed the concentration of the pyridine neucleotide to 10 - 30% of control. To date only one time period has been examined: 4 hours post-dose. (The islet isolation period takes another 90 minutes). It has also been determined that parenteral nicotinamide, 500 mg/kg, IP brings about a striking elevation of insular NAD at four hours - and that the same dose can prevent the marked depression of nucleotide caused by streptozotocin. On the otherhand, studies of islets exposed *in vitro* to streptozotocin have not been clear cut, i.e., no consistent or significant depression of NAD has been seen, even following exposure to streptozotocin at 1×10^{-2} M. However, these studies have been hampered by the rapid and obvious decomposition of streptozotocin at the pH of 7.4 which is required to maintain the islets. Needless to say, a similar pH and therefore, a similar rate of decomposition ought to prevail *in vivo* so that it is not impossible that the failure to depress insular NAD *in vitro* simply indicates that streptozotocin has not been "activated" *in vitro*.

Significance to Bio-medical Research and the Program of the Institute

It is reasonable to hope that powerful and irreversible inhibitors of L-asparagine biosynthesis will confer sensitivity, to L-asparaginase, on tumors naturely resistant to the enzyme, or will prevent the emergence of resistance. It is important to understand the mechanism of action of streptozotocin and the mechanism of interaction of nicotinamide with streptozotocin since these drugs offer one of the most striking examples in the chemotherapeutic armamentarium of an intoxicant and its specific antidote.

Planned Course

Purification of pancreatic L-asparagine synthease and agouti L-asparaginase will continue, hopefully unto homogeneity; further inhibitors of tumoral L-asparagine synthetase will be sought, synthesized and tested. (But more

rational candidate inhibitors are badly needed). Studies of the alkylation of DNA by streptozotocin, just now beginning will be pursued vigorously in the next year.

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3. Schaeppi, U., Heyman, I. A., Fleischman, R. W., Rosenkrantz, H., Ilievski, V., Phelan, R., Cooney, D. A., and Davis, R. D.: Cis-dichlorodiammine-platinum (II) (NSC-119,875): Preclinical Toxicology Evaluation of I.V. Injection in Dogs, Monkeys, and Mice. Cancer Chemother. Rep., 1972 (IN PRESS).

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5. Cooney, D. A., Moore, A. L., Zhagats, R.: A Colorimetric Technique for the Demonstration of L-Asparaginase in Electropherograms. Clinica Chimica Acta, 1972 (IN PRESS).

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8. Fleischman, R. W., Baker, J. R., Schaeppi, U., Thompson, G. R., Rosenkrantz, H., Cooney, D. A., and Davis, R. D.: Azaserine Induced Mandibular Salivary Gland Pathology in Dogs. Toxicology and Applied Pharmacology, 1972 (IN PRESS).

Serial No. NCI-6106
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effect of Antineoplastic Agents on Reproduction

Previous Serial Number: Same

Principal Investigator: Robert L. Dixon and Insu P. Lee

Other Investigators: Michael Innis

Cooperating Units: None

Man Years:

Total:	2 1/2
Professional:	1 1/2
Others:	1

Project Description:

Objectives:

IN VIVO

1. To study the effect of antineoplastic agents on male fertility using serial mating of mice.

2. To obtain fertility profiles by plotting percent fertile mice against days following drug treatment for various agents with known mechanisms of action.

3. To determine if the fertility pattern and spermatogenic cell type affected can indicate the mechanism of action of various drugs.

4. To evaluate the mutagenic potential of anticancer agents.

IN VITRO

1. To describe the individual spermatogenic cell types which are capable of utilizing thymidine, uridine, and L-leucine as an indication of DNA, RNA, and protein synthesis.

2. To describe the effects of various antineoplastic agents on the cellular utilization of these substrates.

3. To study the duration of any drug-induced effects.

4. To correlate these observations with in vivo fertility studies on antineoplastic drug treated male mice.

Methods Employed:

IN VIVO

Serial mating of male mice treated with drugs was used to assess the biological functionality of sperm cells and to produce fertility patterns which have been shown to be inversely related in time to the phase of spermatogenesis damaged by the drug. For instance, drug effects on spermatozoa appear first, and those due to interference with spermatogonia appear last.

IN VITRO

The modified method of Lam, Furrer, and Bruce which allows one to separate various spermatogenic cell types by velocity sedimentation is being used. Testicular cells are prepared by removing the tunica albuginea from the testes under a stereomicroscope, and cutting the testicular tissue into short segments. This tissue is washed into a Nalgene tube with 10 mls of Spinner solution containing 0.5% bovine albumin. Subsequently, the cells are suspended by gently pipetting the suspension up and down with a Nalgene pasteur pipette. Following this, the heavy tissue segments are allowed time to settle. The supernatant of the cell suspension is filtered through a lens paper to remove cell aggregates. Cell number is then determined in a cell counter. Two x 10^7 cells are loaded into the sample vessel and 720 mls of a 1-3% linear gradient of bovine serum albumin in Spinner solution is developed through the bottom of the chamber. At the end of 4 hours of sedimentation, the chamber is drained from the bottom at the rate of 10 mls per minute. One hundred thirty-five individual fractions are collected with an LKB fraction collector. Each fraction corresponds to a sedimentation distance of 0.76 mm. The chamber size is such that 1 mm of vertical distance contains 7.2 mls of gradient fluid.

Following cell fractionation, each sample is filtered and the cells collected on a Millipore Filter (0.5 μ pore size), and the acid insoluble fraction of nucleic acids and proteins is obtained. Each fraction is dried under a hood and then digested with 0.5 mls of solubilizer at 50°C in a mechanical convection oven for two hours. After cooling the vials, 10 mls of toluene containing Liquifluor is added to each vial. The radioactivity in the acid insoluble portion of each fraction is then determined.

To determine the volume of individual cells, an Intertechnique Didac multichannel analyzer was interphased with a Celloscope which analyzed the pulse height signal for cell volume.

The morphology of the cells in each fraction was examined by the exfoliative cytological technique which also allows the cells on each slide to be counted. An ultraphot II Zeiss camera microscope was used to prepare photomicrographs of individual slides.

The major portion of this year's research was related to spermatogenic cell separation and a study of the effects of additional antineoplastic drugs on the incorporation of thymidine, uridine, and L-leucine by various individual spermatogenic cell types. The cellular incorporation of radioactive thymidine, uridine, or L-leucine was studied one hour after intraperitoneal injection of the nucleoside or amino acid. The thymidine peak fraction was identified as spermatogonia which had a sedimentation rate of 5.5 mm/hour. Tritium labelled uridine appeared in the cell fraction sedimenting at 3.9 mm/hour when cells are isolated one hour after injection of the substrate. This peak of activity represents intermediate spermatids. The cell types that incorporated tritium labelled L-leucine one hour after intraperitoneal administration of the amino acid were also identified: the 1.1 mm/hour fraction contained late stages of spermatids and some spermatozoa; the 5.5 mm/hour fraction contained spermatogonial cells; the 8.7 mm/hour fraction represented early spermatids; the 13.1 mm/hour fraction was secondary spermatocytes; and the 17.8 mm/hour fraction was identified as diplotene and pachytene cells.

Three additional antineoplastic agents and one testicular tumorigenic inorganic ion (Cd^{++}) were tested for their effect on the incorporation of thymidine, uridine, and L-leucine. To study in vivo effects of drugs, bleomycin, daunomycin, platinum (diamminedichloro-, cis-) and cadmium chloride were administered intraperitoneally at the maximally tolerated dose. The cell types followed were the spermatogonia, intermediate spermatids and the late spermatids. Groups of treated mice were sacrificed one hour after treatment and also at 7, 14, 21, 28, and 35 days after drug injection. In every group, the thymidine, uridine, or L-leucine was injected one hour prior to sacrifice.

Major Findings:

In Vivo Drug Effects:

1. Bleomycin inhibited DNA synthesis. Functional test of spermatogenic cells by serial mating showed that the early spermatocytes and spermatogonial cells were affected. The biochemical effect, therefore, is well reflected by the functional test. Antifertility effects of this drug are effective as long as 55 days.

2. Daunomycin inhibited thymidine incorporation. Functional test of spermatogenic cells by serial mating test showed only the spermatogonial cells to be affected; this reflects an effect on DNA synthesis.

3. Platinum chloride inhibited thymidine incorporation. Functional test of spermatogenic cells by serial mating test showed only spermatogonial cells to be affected; this reflects an effect on DNA synthesis which occurs only in spermatogonial cells.

4. Cadmium Chloride (Cd^{++}) showed marked inhibition of thymidine incorporation but neither uridine nor L-leucine utilization was affected. Serial mating

studies indicate both spermatids and spermatogonial cells were functionally affected. However, Cd^{++} effects on spermatids were not reflected by L-leucine incorporation while Cd^{++} effects on DNA synthesis are indeed reflected in fertility studies.

Further studies were carried out to identify the cell types which incorporate Cd^{++} and Zn^{++}, and to study the interaction of these two metals. ^{109}Cd and ^{65}Zn were used to monitor the cellular incorporation of these elements. Following intraperitoneal injection of either Cd^{++} or Zn^{++}, peak radioactivity was determined in the spermatogenic cell fraction which sediments at 2.5 mm/hour (spermatids). Similar results were obtained following in vitro incubation of seminiferous tubules with either metal. At equimolar concentrations, Cd^{++} incorporation was 2.5 times faster than the rate of Zn^{++} incorporation. Competitive inhibition could be demonstrated. It has been speculated that spermiogenic cell differentiation requires Zn^{++}. Therefore, effects of Cd^{++} on spermiogenic cells as indicated by fertility studies can probably be attributed to retarding the Zn^{++} uptake into critical spermatogenic cells.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of the effects of antineoplastic agents on reproduction in the past have centered largely on teratogenicity. Few investigations have been concerned with the effect of these drugs upon fertility. However, as more effective drugs and therapeutic regimens have been developed, questions concerning the effect of anticancer agents on reproduction are becoming clinically important.

Van Thiel and DeVita have recently documented the pronounced effect of an antineoplastic drug combination on male spermatogenesis. Seven patients in remission after receiving chemotherapy for lymphoma were evaluated 1-5 years after treatment. Persistent azoospermic men showed complete absence of all germinal elements. However, Sertoli cells and Leydig cells were present. In addition to the clinical aspects, spermatogenesis appears to present an interesting population of cell types for basic studies dealing with drugs that effect cell multiplication. Spermatogonial cells mature in a highly synchronous fashion into only one type of differentiated cell, the spermatozoan. It also should be pointed out that the spermatogenic cell population provides an excellent model system to study the effect of drugs on cells that are not dividing (spermatids and spermatozoa). Spermatogenesis might be a valuable test system to screen for drugs specifically effective against nonproliferating cell populations.

Planned Course:

These studies will be continued and expanded to assess carefully the effects of antineoplastic agents on reproduction. Attempts will be made to correlate these data with clinical findings. Future investigations will include an evaluation of the mutagenic potential of oncolytic agents using the "dominant lethal" technique, and also a study of the effect of these chemotherapeutic

drugs on ovarian function. In addition, the value of rodent spermatogenesis as a means of testing for drugs effective against nonproliferating cell populations will be further investigated.

Publications:

1. Lee, I. P. and Dixon, R. L.: Antineoplastic Drug Effects on Spermatogenesis Studied by Velocity Sedimentation Cell Separation. Toxicol. and Appl. Pharmac., in press, 1971.

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3. Lee, I. P. and Dixon, R. L.: Effects of Vincristine on Spermatogenesis Studied by Velocity Sedimentation Cell Separation and Serial Mating. J. Pharmacol. Exp. Ther., in press, 1971.

Serial No. NCI-6110
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1971 to June 30, 1972

Project Title: The Pathology of Drug-Induced Alterations in Animals and Man

Previous Serial Number: Same

Principal Investigator: David M. Young

Other Investigators: Gerhard R. Krueger
Louis M. Buja
Roger S. Jaenke

Cooperating Units: Laboratory of Pathology, General Laboratories and Clinics, National Cancer Institute
Colorado State University

Man Years:
Total: 2
Professional: 1
Other: 1

Objectives:

The objectives of this project are:
1) To conduct scientific investigations designed to elucidate the mechanism(s) of antineoplastic drug-induced pathologic alterations.

2) To provide guidelines to standardize, semi-quantitate, and computerize pathologic evaluation of experimental animals utilized by contractors in preclinical toxicologic testing of antineoplastic agents.

3) To evaluate and record reports of adverse effects of antineoplastic agents in man and correlate these findings with those recorded in test animals.

Principal Findings:

Histopathologic Effects of Antineoplastic Agents in Cancer Patients

During 1971 a retrospective study of 200 cancer patients was conducted with the objectives: (1) to determine the approximate incidence of antineoplastic drug-induced histopathologic alterations in cancer patients treated at the NIH Clinical Center, and (2) to compare these findings with those reported in animals in order to better evaluate the predictability of the preclinical toxicology protocol. The significant limitations of such a study cannot be overemphasized. Variations in age, health status, and treatment between test animals and man severely retard the process of extrapolation and

predictability between species. The organ systems of man generally are compromised significantly by disease, mainly of neoplastic origin, but also due to therapeutic procedures. Additional degenerative, infectious, nutritional, and environmental disorders are usually present. These factors represent a few of the numerous problem areas in determining specific adverse effects due to drug administration in man.

All of the patients included in the aforementioned study were deceased, were afflicted with one or more neoplastic processes, and were autopsied. The age range was 2-89 years with a mean of 40 years. The most frequently administered anticancer agents were vincristine, prednisone, cyclophosphamide, and methotrexate. Those lesions occurring most commonly in different organ systems which were considered to be either directly or indirectly related to administration of antineoplastic agents are as follows:

1) Integumentary hemorrhages and alopecia
2) Lymphoreticular atrophy
3) Bronchopneumonia with pulmonary fibrosis and edema
4) Hepatic fibrosis, esophageal inflammation and ulcerations, gastrointestinal ulceration and hemorrhage.
5) Renal necrosis and inflammation, hemorrhagic cystitis with epithelia dysplasia
6) Bone marrow atrophy
7) Testicular atrophy, aspermatogenesis and ovarian cellular atypia.

Over one-half of the patients studied were considered to exhibit well documented septicemia terminally. The most frequently isolated microorganisms were Streptococcus, Escherichia coli, Pseudomonas, Klebsiella, and Candida.

In summary, specific lesions observed in cancer patients which appear to be related to cancer chemotherapy are:

1) Cyclophosphamide-induced hemorrhagic cystitis
2) Bleomycin-induced pulmonary fibrosis
3) L-Asparaginase-induced pancreatitis
4) Daunomycin-induced degenerative myocardiopathy
5) Methotrexate and 6-Mercaptopurine-induced hepatic fibrosis
6) Generalized septicemia with disseminated infection

Of the above, preclinical toxicologic studies in the dog and monkey did not predict for numbers 3, 4, or 6.

Standardization of Pathologic Evaluation of Dogs and Monkeys Utilized for Preclinical Toxicologic Testing of Antineoplastic Agents

During the past year it became evident increasingly that a standardized system of reporting pathologic alterations in test animals was urgently needed. Therefore, an attempt to standardize, semi-quantitate and computerize pathology reporting by contractors of the Laboratory of Toxicology was developed in a system entitled "Standardized Toxicologic Pathology". This system utilizes four-digit code designations for anatomical structures and pathologic changes. Lesions are graded according to degrees of severity

(I through IV); whereas, changes in clinical parameters are rated 1 through 9. A keysort punch card is provided for the pathology report on each test animal. Upon termination of preclinical toxicologic studies of an antineoplastic agent, the completed keysort cards are returned by the contractor to the Laboratory of Toxicology. The information on the keysort cards is transferred to computerized tape and the cards filed in the Comparative Pathology Section of the Laboratory of Toxicology.

In addition to standardization of pathology reporting, the contractors of the Laboratory of Toxicology have been provided with guidelines for necropsy of the dog and monkey, proper tissue selection, fixation and staining procedures, copies of canine and simian gross pathologic records and lists of recommended necropsy instruments and appropriate references.

This new system of standardization is currently in the process of being implemented by the contractors of the Laboratory of Toxicology.

Evaluation of Reports of Adverse Effects of Antineoplastic Agents in Man and Animals

Unpublished and published data is reviewed periodically to determine the current spectrum of adverse effects of anticancer agents. Copies of clinical records and autopsy protocols on cancer patients treated at the NIH Clinical Center are reviewed on a weekly basis. Notations are made concerning drug-associated lesions and the reports are filed with a list of antineoplastic agents administered attached to the face page of the autopsy protocol. In addition, monthly "NCI Summaries of Death and Complications" are reviewed for drug-associated unexpected findings. Published reports of adverse effects of antineoplastic agents are reviewed with pertinent information transferred to keysort punch cards which are in turn filed. Many of these reports involve use of antineoplastic agents in non-neoplastic conditions, e.g., psoriasis, arthritis, glomerulonephritis and organ transplantation. The above findings are correlated with reported alterations occurring in dogs and monkeys utilized in preclinical toxicologic studies.

Daunomycin/Adriamycin-Induced Cardiomyopathy

Daunomycin and Adriamycin are being used currently to suppress and obliterate a wide range of neoplastic processes. Recent reports reveal that Adriamycin is active against bone tumors in man. With the increase in use of these two agents, more reports of life-threatening cardiotoxicity have been received. Unfortunately the cardiomyopathic potential of Daunomycin/Adriamycin was not predicted for by preclinical animal toxicologic studies. Therefore, present investigational efforts are directed to defining an animal model that exhibits the delayed clinicopathologic alterations observed in man with Adriamycin. Currently, miniature swine and rabbits are being employed to assist in elucidation of the mechanisms involved in Daunomycin/Adriamycin-induced cardiotoxicity.

Clinical congestive heart failure is the most consistent sign of cardiotoxicity in man. Histopathologic evaluation of myocardium has usually revealed only

subtle, non-specific findings. Recent ultrastructural studies of heart muscle from patients at the NIH Clinical Center exhibiting cardiotoxicity have revealed some striking alterations. The destructive influences of Daunomycin/Adriamycin appear to be directed towards myocardial subcellular structures. Drs. Krueger and Buja are collaborating in performance of these studies. In addition, Dr. Roger Jaenke, Colorado State University (Contract No. NIH 71-2192) is the principal investigator involved in designing the animal studies.

The Pathogenesis of L-Asparaginase-Induced Hypocalcemic Tetany in Rabbits

A number of hypocalcemic agents have been employed experimentally to study the endocrine function of the parathyroid glands. None of these agents have been reported to produce its effect by selective destruction or interuption of the secretory cycle of the parathyroid chief cell. This investigation was designed to determine whether L-Asparaginase (Asnase) toxicity in the rabbit represented such a selective endocrine cell destruction.

The I.V. administration of 1,000 I.U. of Asnase/kg/day to 6 week old rabbits resulted in fatal hypocalcemic tetany in 70% of the animals within 10 to 51 hours. In addition, tetanic rabbits exhibited hyperphosphatemia, hypomagnesemia, hyperkalemia and elevated blood urea nitrogen levels. Histopathologic evaluation of internal and external parathyroid glands (PTG's) revealed diffuse multifocal areas of necrotic chief cells. Electronmicroscopically chief cells were shrunken, devoid of electron-dense secretory granules, contained irregularly shaped nuclei and cytoplasmic autophagic vacuoles. Determinations of L-asparagine concentration in the PTG's revealed a significant reduction compared to controls. Protein synthesis in short term explants of external PTG's was inhibited markedly by Asnase. L-Asparagine synthetase activity in normal rabbit PTG's was found only in low levels which correlates well with the sensitivity of the gland to Asnase. Prophylactic (parathyroid extract) and therapeutic (calcium gluceptate) treatments were successful in alleviating the fatal hypocalcemia in a significant number of rabbits. Surgically thyro-parathyroidectomized rabbits developed similar overt signs and clinicopathologic manifestations as Asnase-treated animals with acute death. Therefore, the administration of Asnase to rabbits appears to result in alteration of parathyroid hormone synthesis, storage and/or secretion with development of an acute hypoparathyroid status.

Significance to Bio-Medical Research and the Program of the Institute:

The need for a better understanding of the pathologic effects of anticancer agents cannot be overemphasized. Upon elucidation of the pathogenesis of drug toxicity, methods can be explored to nullify the adverse effects while retaining the drug's anticancer activity. A by-product of these studies of drug-induced lesions is the possible definition of new experimental models to study spontaneous disease in animals and man. For example, Daunomycin/Adriamycin-induced cardiotoxicity may prove to be an excellent model to study the pathogenesis of congestive heart failure; L-asparaginase-induced hypocalcemic tetany in the rabbit may provide the first experimental model of selective parathyroid cell destruction by a chemical agent to study the

mechanisms of calcium homeostatis; and Bleomycin-induced integumental and pulmonary lesions may provide an excellent model for experimental studies of connective tissue diseases.

Comparison of drug-induced lesions in man and animals will assist in the critical evaluation of predictability for specific adverse effects utilizing a carefully designed preclinical toxicologic protocol. In addition, standardization of reporting pathologic effects of anticancer drugs will expedite transfer of agents through the Laboratory of Toxicology in the Linear Array of the Chemotherapy Program.

Proposed Course:

The present investigations will be continued and expanded to better assess the pathologic effects of anticancer agents. Emphasis will be directed towards defining mechanisms of delayed toxicity that seem to plague the successful use of several of the more promising cancer chemotherapeutic agents (Bleomycin, Adriamycin, Daunomycin, BCNU, CCNU). In addition, a quality control checking system will be implemented to assess the performance of contractors of the Laboratory of Toxicology in pathologic evaluation.

Publications:

1. Young, D. M. and Capen, C. C.: Calcitonin Activity in Ultimobranchial Neoplasms from Bulls. Vet. Path. 8, (1971): 19-27.

2. Young, D. M., Capen, C. C., and Black, H. E.: The Effect of a Low Calcium Diet and Vitamin D on Thyrocalcitonin in Cows. Endocrinology 90, (1972): 240-245.

3. Young, D. M. and Capen, C. C.: The Fine Structure of Thyroid Parafollicular Cells from Cows Fed a Low Calcium Diet and Vitamin D. Virchows Arch. Zellpath. 8, (1971): 288-298.

4. Primack, A., Young, D. M., Homan, E. R.: Multiple Congential Anomalies in Rhesus Monkey: A Case Report. Teratology, Vol 5, April 1972, 137-141.

Serial No. NCI-6113 (New)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1971 to June 30, 1972

Project Title: The Detection, Quantitation, Evaluation, and Prediction of the Pathologic Effects, Especially on the Hematic System, of the Toxicity of Antineoplastic Agents and Studies of Other Conditions of Host-Defense Failure

Previous Serial Number: None

Principal Investigator: David J. Prieur

Other Investigator: David M. Young

Cooperating Units: Chemotherapy Program

Man Years:
Total: 2
Professional: 1
Others: 1

Objectives:

1. To detect, quantitate and evaluate the pathologic effects of anticancer agents.

2. To elucidate the mechanisms of these toxic effects.

3. Relate the toxic effects on the hematic system to the host-defense failure in patients treated with the agents.

Project Description:

During the past year a laboratory was equipped, personnel recruited, and studies initiated to detect quantitate, evaluate, and predict the effects of antineoplastic agents on the hematic system. Studies, utilizing etiocholanolone and Piromen, to measure bone marrow reserve in dogs have been performed, and studies of the effects of various antineoplastic agents upon some of the *in vitro* parameters of host-defense have been initiated.

Studies defining and semi-quantitating a defect in the function of renal lysosomes in mice with the Chediak-Higashi syndrome were completed. Ultrastructural studies on platelets from cattle with the same disease are in progress.

A protocol for the pathologic evaluation of dogs and monkeys utilized for the preclinical toxicology of candidate antineoplastic agents was prepared in cooperation with Dr. David M. Young.

An ultrastructural study of various fractions of thymocytes from AKR mice was performed in collaboration with Drs. N. Omine and S. Perry and an ultrastructural evaluation of the effects of the antineoplastic agent, o,p'-DDD was conducted with Dr. Michael Hart. An electron microscopic study of the effects of the anticancer agents, Procarbazine and Vincristine, upon the morphology of sperm was performed in collaboration with Dr. Insu Lee, and an ultrastructural assay of various cultures of L1210 leukemia cells for virus was performed in collaboration with Dr. Kurt Kohn.

Major Findings

The Pseudomonas polysaccharide Piromen appears to be useful to measure the bone marrow reserves of dogs whereas the androgen etiocholanolone appears much less useful for such studies. These investigations are still in progress and are subject to future revision.

The renal lysosomes of mice with the Chediak-Higashi syndrome were found to catabolize the exogenous enzyme, horseradish peroxidase, at a slower rate than normal mice. An ultrastructural evaluation of platelets of cattle with the same syndrome revealed no morphologic abnormalities of the lysosomal granules but revealed instead a decreased number of osmiophilic dense bodies which could not be increased upon incubation with serotonin.

Significance to Bio-Medical Research and the Program of the Institute:

Most of the anticancer agents have adverse effects upon the bone marrow of treated patients and it has also been shown that such treated patients are quite susceptible to bacterial and fungal infections. In fact some of the patients who are cured of cancer die from infections to which they are susceptible because of the anticancer agents. It takes approximately 5 days before toxicity to the bone marrow is expressed in the numbers of circulating white blood cells. If a method of detection of toxicity based upon the number of cells in the bone marrow reserve can be perfected the toxicity will be recognizable sooner so that appropriate measures can be taken.

The Chediak-Higashi syndrome is a genetic disease which has as one of its manifestations, an increased susceptibility to infection. Morphologic abnormalities of lysosomes are present and the demonstration of defective catabolism of exogenous material by the renal lysosomes suggests a similar

defect in other cell types. A similar defect in the lysosomes of the neutrophils would almost certainly result in an increased susceptibility to infection. In the Chediak-Higashi syndrome there is also an in vivo bleeding tendency but no abnormalities of the in vitro clotting factors. The role of platelets in blood coagulation is well known. In spite of the fact that platelets take up and store large amounts of serotonin no role for serotonin in the function of platelets in blood coagulation has ever been recognized. This report of the first disease with a qualitative platelet disorder associated with a defect in serotonin metabolism may allow a opportunity to elucidate the role of serotonin in blood coagulation and platelet function. In addition the demonstration that the lysosomal granules are morphologically normal but that the small dense granules are functionally abnormal in platelets of cattle with the Chediak-Higashi syndrome casts some doubt on the definition of the syndrome as a lysosomal disease.

The protocol for the pathologic evaluation of dogs and monkeys in preclinical toxicology establishes a standardization of procedures for the contractors of the Laboratory of Toxicology. This protocol will increase the precision and accuracy of the contractors in the reporting of the lesions in animals and will increase the efficiency of prediction for toxic manifestations of antineoplastic agents administered to cancer patients.

Future Work

Each of these areas of research will be continued and expanded within the Laboratory of Toxicology the coming year.

Publications:

1. Prieur, D. J., Davis, W. C., and Padgett, G.A.: Defective function of renal lysosomes in mice with the Chediak-Higashi syndrome. Am. J. Pathol. 67: 215-228, 1972.

Serial No. NCI-6114 (New)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Progress Report
July 1, 1971 through June 30, 1972

Project Title: Effect of Prenatal Exposure to Antineoplastic Agents on Gonadal Function in the Offspring of Rodents.

Previous Serial Number: None

Principal Investigator: John A McLachlan and Robert L. Dixon

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	1 1/2
Other:	0

Project Description:

Objectives:

1. To evaluate the effects of prenatal exposure to antineoplastic agents on the subsequent reproductive capacity of the offspring.

2. To investigate the mechanisms involved in the production of subfertility in adults as a result of their *in utero* exposure to anticancer agents.

3. To assess the transplacental carcinogenic potential of various antineoplastic drugs.

4. To study dose-response relationships and sensitive periods for the interaction of anticancer drugs and the fetal gonad.

Methods:

The most sensitive measurement of female gonadal function is her total reproductive capacity as determined by repetitive forced-breeding techniques. When these techniques are coupled with the determination of ovarian periodicity by daily vaginal smears as well as histolopathological evaluations of the ovary, including quantitative determinations of the

various follicular classes, an estimate of the functional status of the female gonad may be obtained. Moreover, methods involving discontinuous gradient polyacrylamide gel electrophoresis with differential staining are being developed to help evaluate possible biochemical lesions in the ovary as a result of prenatal treatment with antineoplastic drugs.

Major Findings:

These studies were initiated with procarbazine (NSC-77213) and cyclophosphamide (NSC-26271); the compounds were given to pregnant mice in relatively low doses (20 mg/kg, and 10 mg/kg, respectively) which were 1/10 those associated with gross congenital malformations. The drugs were given as a single dose on either the 10th (pre-DNA synthetic phase of the oocyte), 12th (optimal period of oocyte DNA synthesis), or 17th (post-DNA synthetic phase of the oocyte) day of gestation.

Such prenatal treatments had no effect on the fertility of the male offspring which was evaluated when they attained sexual maturity. However, during the first month of forced-breeding studies, the sexually mature female mice exposed to either antineoplastic agent *in utero* produced fewer than half as many offspring as their corresponding controls. The most significant effect was seen in those mice treated on the 12th day of pregnancy. This apparent subfertility could not be explained by differences in estrous cycles, ovarian morphology, responsiveness to exogenous gonadotropins, or mating behavior. The prenatally treated females are currently in their 4th month of forced-breeding experiments and will be continued throughout their reproductive lifetimes.

Further studies have shown that the degree of morphologically detectable damage following *in utero* exposure to these compounds is dose-related. For example, following administration of 400 mg/kg of procarbazine on the 12th day of gestation, histologically detectable alterations became apparent in only one week.

In addition, large groups of mice from the prenatal treatment studies are being maintained, with representative animals sacrificed at 3 month intervals and examined histologically, in order to evaluate the transplacental carcinogenic potential of these doses of procarbazine and cyclophosphamide.

Experiments are currently underway with high resolution polyacrylamide gel electrophoresis combined with differential staining for different protein classes as well as enzymatic staining techniques in order to develop tools to assess biochemical lesions in the ovaries of animals exposed to antineoplastic drugs.

Significance to Biomedical Research and the Program of the Institute:

Although many compounds are continuously introduced into our environment, few of them have been examined for their potentially toxic effect on reproduction and development. Moreover, virtually nothing is known about the

effect of prenatal exposure to common drugs and chemicals on the postnatal development of the offspring.

The fact that no division of oocytes occurs postnatally in man or laboratory rodents makes the process of oogenesis especially susceptible to chemical intervention during the prenatal period. However, the effects of such *in utero* drug exposure may not become evident until much later in the animal's life when sexual maturity is reached. A well-known example is the recently reported incidences of vaginal cancer in young women whose mothers had been given diethylstibestrol during their pregnancy.

The antineoplastic agents represent classes of drugs which are known to exert potent effects on rapidly dividing or differentiating tissue suggesting that these compounds may exert a toxic effect on the developing gonad. In addition, such studies may provide insights into the possible long term genetic consequences of the exposure of mammalian oocytes to cyto- and nucleotoxic agents.

Planned Course:

These studies will be continued and expanded to include a wider range of clinically useful antineoplastic agents as well as evaluations of the relationships of drug dose and time of administration to the gonadotoxic effects. Moreover, the localization of such compounds in the fetal and adult ovaries is under investigation. Concomitant studies to develop *in vitro* systems for the determination of drug effects on oogenesis as well as ovarian steroid biosynthesis will be initiated.

Serial No. NCI-6115 (New)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Possible Mechanism of Ellipticine Induced Hemolysis of Human and Rhesus Monkey Red Blood Cells

Previous Serial Number: None

Principal Investigator: Insu P. Lee

Other Investigators: Michael Innis

Cooperating Units: None

Man Years:

Total:	2
Professional:	1
Others:	1

Project Description:

Ellipticine (NSC-71795) an alkaloid extracted from two botanically related Australian plants has been tested by Drug Research and Development (DR&D) of the National Cancer Institute and found active against Sarcoma 180, Adenocarcinoma 755, and Lymphocytic Leukemia L1210. Cytotoxicity has been demonstrated against human carcinoma in cell culture (KB) and subsequently several methods have been developed for the synthesis of ellipticine and for several ellipticine analogs. In addition, it has been reported that the drug inhibits DNA, RNA, and protein synthesis in human embryonic fibroblasts in vitro.

On the basis of its antineoplastic activity in rodents, preclinical toxicology was undertaken to assess the feasibility of initiating clinical trials with the drug. During the course of these studies, it became apparent that ellipticine when administered intravenously to either Rhesus monkeys or Beagle dogs at doses as low as 3 mg/kg caused severe hemolysis.

The present investigation is concerned with a possible mechanism and factors affecting ellipticine-induced hemolysis of human and Rhesus monkey red blood cells.

Objectives:

In Vitro and In Vivo Studies:

1. To determine, in vitro and in vivo, the degree of hemolysis occurring with varying concentrations of ellipticine.

2. To ascertain the possible mechanisms of hemolysis.
3. To study factors affecting the ellipticine-induced hemolysis.
4. To determine, in vitro and in vivo, agents which will prevent ellipticine-induced hemolysis.

Methods:

Standard methods were used to prepare human red blood cell suspensions, varying osmolarities of NaCl solutions, and to determine osmotic fragility. Surface activities of varying aqueous concentrations of ellipticine were determined by means of the ring method using the Cenco-duNouy surface tensiometer. The mean cellular volume of red cells was estimated with a Celloscope interphased with a multichannel pulse height analyzer. Ellipticine was determined at 303 nm with a Gilford spectrophotometer. The extinction maximum of the ultraviolet spectrum of ellipticine in 0.1 N HCl was 303 nm and the extinction coefficient was 51,500. Circular difchroism spectra was taken with a Cary 60 spectropolarimeter. Anesthetized Rhesus monkeys received various formulations of ellipticine during in vivo studies, and hemochromogen was determined by the benzidine method.

Major Findings:

Ellipticine as either the HCl salt or the polyvinylpyrrolidone formulation obtained from DR&D caused severe hemolysis in vivo. However, the citrate formulation at all doses tested, showed no increase in the plasma hemochromogen level.

The extent of hemolysis induced by ellipticine and ellipticine analogs correlates well with their surface tension ($r = 0.89$). However, neither the degree of hemolysis nor surface tension appears to correlate well with experimental antitumor activity.

11-Demethylellipticine and 9-methoxy-ellipticine were the least hemolytic ellipticine derivatives.

The mechanism of hemolysis is due to excessive accumulation of ellipticine bound to the lipid component of the red cell membrane and possible alteration of the lipoprotein conformation of membranes. This conclusion is based on the distribution of the ellipticine in lipid, protein, and hemoglobin as well as circular dichroism spectras.

Several calcium chelating agents inhibited the absorption of ellipticine to the red cell membrane. Furthermore, citrate inhibition of ellipticine binding is noncompetitive.

Significance to Bio-Medical Research and the Program of the Institute:

The hemolytic potential of ellipticine would have essentially prevented further preclinical toxicology and eventual clinical introduction unless ameliorated. These studies present valuable basic information concerning the mechanism of this drug-induced hemolysis and formulation procedures that can be used to avoid the problem.

Planned Course:

These studies are to be completed during this fiscal year.

NSC-3053 Actinomycin D South Shore Analytical & Research Lab.,Inc.
NSC-109,229 L-Asparaginase

Actinomycin D was given four hours before L-asparaginase (EC-2) in the present study in the hope of preventing the induction of L-asparagine synthetase and the subsequent emergence of resistance to L-asparaginase. The toxicity of the two drugs was probably not synergistic. Several other findings emerged. Intravenous doses of Actinomycin D at the level of 0.04 mg/kg/day given daily for five days was the lethal dose whereas 0.015 mg/kg/day given over the same period was a low toxic dose in one of the monkeys. L-Asparaginase given alone produced BSP retention in both recipients. However, 4 and 5 days after the termination of therapy, fatty liver was found in only one of these monkeys. This then is either a rare example of a monkey not showing steatosis after therapy with high doses of L-asparaginase or an unusually rapid resolution of the fatty lesion.

NSC-58514 Chromomycin A3 Mason Research Institute, Inc.

Toyomycin is the Japanese clinical formulation of Chromomycin A3. The data given below summarize the comparative toxic properties of Chromomycin A3 formulated at Ben Venue Laboratories and Toyomycin formulated in Japan. This comparison study has established that both formulations produce essentially the same derangements which are described below. Both the Rhesus monkey and Beagle dog showed anorexia, emesis, and diarrhea after high doses of the drug. Inflammation and necrosis of the epithelium of the gastrointestinal tract were the histopathologic correlates of these clinical effects. Hypoplasia and necrosis of the marrow were detected in both species; monkeys became leukopenic promptly, whereas, the effect tended to be delayed in dogs. Both formulations damaged lymphocytes and plasma cells. Both species exhibited azotemia after either formulation, occasionally after low doses of drug. The renal tubules of the monkey and dog often exhibited necrosis following treatment with both formulations. The reversibility of renal lesions was not definitely established. In the monkey especially, rather regular significant depressions of serum calcium were produced by both formulations. Occasionally elevations of phosphate accompanied these depressions. Thus, as it is studied further, Chromomycin resembles its close congener, Mithramycin, in this respect.

NSC-68075 Thalicarpine Arthur D. Little, Inc.

Thalicarpine administered intravenously to Rhesus monkeys produced toxic effects which were similar for all dosage regimens tested but were not dose-related. When death occurred, it was always within a few minutes of drug administration and was poorly correlated with dose level. The syndrome preceding death consisted of progressive signs of acute toxicity. Salivation, lacrimation, injected sclerae and mydriasis, sometimes marked in severity, accompanied dyspnea and Cheyne-Stokes respiration. These signs were followed by tonic convulsions, apnea, and cardiac arrest. Elevations of SGOT and SGPT, never more than moderate in severity, tended to be sporadic although sometimes persistently recurrent. Hepatocytopathology was reflected in cloudy swelling and glycogen depletion, possibly concentrated in the centrilobular area. Elevation of BUN was a commonly observed chemical alteration. Irregular electrolyte changes and hypoglycemia might also reflect altered renal function.

Acute focal interstitial nephritis, glomerular congestion and proximal tubular precipitate were histological findings. Slight to moderate erythroid hypoplasia was observed, but not uniformly, and severe lymphoid depletion was observed only in one animal held several weeks after treatment. Anemia was noted in both vehicle control and test animals. The changes in blood chemistry, clinical observations, and pathology seen in these studies parallel closely those seen previously in the studies with dogs. Single intravenous "push" doses of Thalicarpine produced deaths in monkeys on day one at all doses tested. These deaths are apparently related to an effect of the drug on the cardiovascular-respiratory system. Animals survived fourteen daily doses of 5.2 mg/kg and ten doses of 10.4 mg/kg given two courses of daily treatment for 5 days separated by a 9-day rest period. The erratic nature of the observed acute deaths associated with Thalicarpine suggest that the rate of injection and the concentration of drug are important considerations. On-going studies have indicated that monkeys survive doses, given by infusion, that are lethal when given by "push". It is especially important that the clinician be aware of possible cardiovascular-respiratory hazards associated with Thalicarpine in addition to the usual organ toxicities associated with oncolytic agents.

NSC-95,441 Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso- Southern Research Institute

Methyl CCNU

Three nitrosourea derivatives have so far been proposed for clinical trials based on their dramatic effectiveness against L1210 leukemia and other transplantable tumors. These three drugs are: NSC-409,962, 1,3-bis(2-chloroethyl)-1-nitrosourea, BCNU; NSC-79,037, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, CCNU; and NSC-95,441, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, MeCCNU. Each of these drugs has undergone extensive preclinical toxicology while NSC-409,962 and NSC-79,037 currently are used clinically. All of these agents have essentially similar preclinical toxicity.

Severe marrow hypoplasia in both dogs and monkeys is observed at high doses of these drugs. Reversible leukopenia and anemia generally occur in animals surviving initial insult. Delayed thrombocytopenia is also observed, but is reversible. Atrophic and hemorrhagic lymph nodes are noted in both the dogs and monkeys administered higher doses. Delayed liver damage is the principal dose-limiting toxicity in dogs. It is expressed by the sudden appearance of markedly elevated transaminases, alkaline phosphatase and BSP levels. There is an inverse relationship between the size of the dose and the length of time that elapses before the first elevation of transaminases is recorded. Renal toxicity is evident in both species, but is more marked in the monkey. Dogs show elevated BUN levels and congestion of renal corticomedullary junctions. Monkeys treated with single oral doses of MeCCNU greater than 50 mg/kg had severe and seemingly irreversible renal tubular (proximal convoluted) damage. The development of this lesion is usually associated with a progressive, fatal uremia. Large toxic doses also produce vomiting, mucosal desquamation, and hemorrhage. Several animals receiving large toxic doses of drug demonstrated pulmonary edema, myocardial infarcts, and subendocardial and pericardial hemorrhages. These findings were more prominent in dogs than in monkeys.

NSC-102,627 1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride — South Shore Analytical & Research Lab., Inc.

NSC-102,627 is an exceedingly powerful and specific myelotoxin. When given on a weekly schedule, high doses of the drug selectively damage all the formed elements of the bone marrow - without significant primary toxicity to other organ systems. When the marrow is insulted on a weekly basis, it is slow to recover, but eventually (four weeks or so after the final injection of the agent) the erythroid, myeloid, and thrombocytic elements regain their approximate pretreatment levels. No testicular abnormalities were detected in the present study in the rhesus monkey nor were the previously reported testicular changes seen in dogs given large doses of this dimethanesulfonate reproduced in the present study.

NSC-102,816 s-Triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl- — Arthur D. Little, Inc.

5-Azacytidine

The intravenous toxicity in Beagle dogs of the clinical preparation of 5-Azacytidine containing PVP was compared with the toxicity of bulk drug in distilled water. Each formulation or control saline was administered as two courses of five daily doses separated by an interval of nine days. Changes in the drug-treated dogs were generally mild and reversible or similar to changes seen in control animals. There were no discernible significant differences between the effects of bulk drug and clinical formulation. The changes seen were qualitatively and quantitatively consistent with those reported in previous toxicologic studies of the bulk drug. Mild anemia was seen in both treatment groups and in control animals. Mild reversible leukopenia was also observed in both groups. One dog given bulk drug exhibited slight erythroid hypoplasia on histologic examination of the bone marrow. Reversible SGPT elevations in the absence of changes in SGOT or alkaline phosphatase are similar to those reported in earlier work using bulk drug. One control animal also exhibited a mild SGPT elevation. Slight congestion of the portal veins and slight to moderate reduction in pericentral glycogen were observed in one dog following administration of bulk drug. Reversible mild BUN elevations observed in dogs receiving both formulations are consistent with earlier results. Renal tubular precipitate seen in treated and control animals is regarded as incidental.

NSC-104,469E Cholest-5-en, 3β-hydroxy-, [p-[bis(2-chloroethyl)amino]phenyl]-, acetate — Mason Research Institute, Inc.

Phenesterin

The oral administration of Phenesterin to Rhesus monkeys every fourth day for fourteen treatments resulted in non-lethal signs of toxicity. Several monkeys

on doses of 50 and 100 mg/kg every fourth day for fourteen treatments exhibited emesis and diarrhea; whereas, those on lower doses survived the treatment in good general condition. The most striking alteration was a slight to severe anemia with macrocytic and hypochromic manifestations. This may have been partly due to the frequency of bleedings. A dose as large as 25 mg/kg every fourth day for fourteen treatments was considered well tolerated. Alterations in functional parameters were reversible and no drug-related pathologic changes were observed at necropsy. Erythrocytic parameters were decreased resulting in the development of a slight to marked anemia. In addition, leukopenia, neutrophilia, and reticulocytosis were observed frequently. The majority of these changes were also observed in vehicle control monkeys and, therefore, are most likely not drug-related. All the treated monkeys exhibited lymphopenia. Histopathologic evaluation of lymphoid tissues revealed depletion and atrophy in only two of the eight monkeys treated with the drug.

NSC-107,392 Picolinaldehyde, 5-hydroxy-, thiosemicarbazone — Mason Research Institute, Inc.

5-HP

Two dogs receiving five daily doses of 43.7 mg/kg were sacrificed two days after treatment in reduced general condition; whereas, all other dogs were sacrificed in good general condition. The most consistent evidence of toxicity was the development of mild anemia and severe leukopenia. At high doses, test animals exhibited emesis, diarrhea, weakness, and weight loss. These changes were consistent with observed alterations in liver function tests (increased alkaline phosphatase levels and BSP retention). Mild anemia and leukopenia were observed in several of the dogs administered a total daily dose greater than 10 mg/kg. Bone marrow alterations oscillated from hyper- to hypo-cellularity with congestion. The spleen showed evidence of extramedullary hematopoiesis in a number of test dogs. Although circulating lymphocyte counts fluctuated widely in dogs on test, only at the highest dose could this alteration be correlated with histologic lymphoid atrophy. The production of dark urine at higher doses was thought to be due to the presence of a chelate which may or may not be associated with elevated BUN levels. At the highest dose these changes were compatible with histopathological findings of nephrosis. The schedule dependency of this drug was evident following treatment every three hours for 24 hours.

NSC-109,229 L-Asparaginase, from *Escherichia coli* — South Shore Analytical & Research Lab., Inc.
NSC-106,977 L-Asparaginase, from *Erwinia carotovora*

L-Asparaginase from *Erwinia corotovora* is significantly less steatogenic than L-asparaginase from *E. coli* (EC 2). The fatty metamorphosis caused by EC 2 is diffuse and intense; the fat globules which accumulated after treatment with *Erwinia* L-asparaginase were most often sketchy, with a midzonal distribution. Both enzymes appeared to alter glucose tolerance in a mild but erratic way: *Erwinia* L-asparaginase showed this effect only at the dose of

10,000 IU/kg/day x 5 days; whereas, EC 2 delayed, in one of three monkeys, the removal of a glucose-load quite significantly after 5 doses of 1,000 IU/kg/day x 5 days. Only high doses of L-asparaginase from Erwinia produced a transient leukopenia. In contrast, low doses of EC 2 depress the number of circulating white blood cells. EC 2 depresses hepatic L-asparagine significantly whereas Erwinia L-asparaginase does not.

NSC-109,724 1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino] tetrahydro-, 2-oxide — Arthur D. Little, Inc.

Iphosphamide

Iphosphamide, an analogue of cyclophosphamide, was administered intravenously to Beagle dogs to assess its toxicity. These single doses ranged from lethal to nontoxic. None of the observed toxicities were unexpected. Dose-related anemia and a fall in white blood cell count and platelets were noted. The highest dose tested (66 mg/kg x 1) was associated with hypoplasia of the bone marrow. Occasional transaminase spikes and BSP retention were recorded. Histopathologic alterations of the liver included bile stasis, glycogen depletion, fatty metamorphosis, and necrosis. Focal necrosis of the epithelial lining was also noted at the highest dose tested. Dose-related hematuria was observed with all doses except the highest not toxic. Reversible elevation of BUN was a common finding at toxic doses. Only at the dose of 66 mg/kg were histopathologic lesions of the kidney and urinary bladder recorded. These were described as acute focal interstitial nephritis, and necrosis of the proximal tubules; the urinary bladder had slightly hemorrhagic subepithelial areas. Focal edema of the lungs was seen at the higher doses. The ratio of the lethal dose to highest non toxic dose is eight, and indicates a relatively shallow dose-response slope for an antineoplastic agent.

NSC-112,259E Estra-1,3,5(10)-triene-3,17β-diol,bis [[p-[bis(2-chloroethyl) amino]phenyl]acetate] — Mason Research Institute, Inc.

The oral administration of NSC-112,259E to Rhesus monkeys resulted in characteristic signs of estrogenic activity. Alterations were manifested by generalized edema, weight gain, and lymphoid atrophy. All monkeys were sacrificed in good general condition. Primary and secondary sex organs of both male and female monkeys responded to the estradiol mustard compound with signs of erythema and edema. Female monkeys exhibited estrus and increased mammary development. Male monkeys developed severe edema of the genital region with phimosis, occasional prostatic metaplasia with accompanying cellular infiltrate and increased mammary development. These alterations were substantiated by histopathologic evaluation of appropriate tissues. This agent appeared to have lymphocytolytic tendencies with resultant lymphoid atrophy in both male and female monkeys. Changes in hematologic parameters included development of anemia, mild sporadic decrease in platelets, leukopenia, reversal of neutrophil to lymphocyte ratios, mild increase in prothrombin time and occasional reticulocytosis. The anemia may be partly due

to frequent bleedings. Decreased serum calcium levels observed occasionally in test monkeys may reflect an estrogenic effect upon bone mineral metabolism. At the highest dose the administration of this oncolytic agent induced emesis and diarrhea. Mild hepatic changes were evidenced at various doses by increased BSP, SGOT, and SGPT levels and infrequent microscopic lipidosis.

NSC-119,875 Platinum, diamminedichloro-, cis — Mason Research Institute, Inc.

NSC-119,875 exhibits potent antitumor activity when given daily to rodents bearing Sarcoma 180, Leukemia 1210, Dunning ascites leukemia or Walker carcinosarcoma. The agent is a powerful inhibitor of DNA synthesis in mammalian tumor cells, but this inhibitory effect is ordinarily fully expressed only after a lag period of from six to twelve hours following drug injection; thereafter, the inhibition persists for at least 96 hours. It has been suggested that, if the persistent inhibitory action on DNA synthesis is directly related to the chemotherapeutic efficacy of this agent, or to one of its metabolic products, an intermittent treatment regimen might be as effective as daily treatment. To date, however, this platinum salt has not been found to exhibit schedule-dependent chemotherapeutic activity, but dividing the daily dose appeared to decrease its toxicity in mice. In the present study, the toxic action of NSC-119,875 was examined in Beagle dogs and Rhesus monkeys. Very high doses of this agent produced prompt and severe emesis, anorexia, abdominal tenderness, and diarrhea in both the dog and monkey. The histopathologic correlate of these clinical signs consists of a severe enterocolitis with occasional examples of damage to the accessory glands of digestion and even to omental fat. The kidney was the principal target for the toxic actions of the platinum compound. At all but the very lowest doses studied, there were examples of alterations of renal function, pathologic urinalyses, or deranged urinary excretion of enzymes. However, the metal produced acute renal tubular necrosis in dogs only at the highest doses studied while only tubular fat depletion or no visible changes were observed at the lower doses. Monkeys responded to the top dose with nephrosis or interstitial nephritis; whereas, monkeys receiving low doses, even those whose renal function was known to be altered on chemical grounds, often exhibited no histopathology. It is possible that the derangements of serum electrolytes seen after high doses in both species played a cooperative role. In both dogs and monkeys, high doses of platinum promptly destroyed circulatory lymphocytes and brought about striking lymphoid atrophy. This action was not detected at lower doses. Despite the occasional elevations of transaminases seen during the monitoring of the clinical chemistry of the treated animals, no significant hepatic histopathology, apart from mild fatty changes, was produced by this agent. Severe hypoplasia of the bone marrow was produced by high doses of the drug in both dogs and monkeys, but this effect was only poorly expressed in the peripheral blood; hematologic monitoring often failed to give evidence of the myelotoxicity of the oncolytic agent. The clinical formulation produced toxicity which was qualitatively similar to that produced by the bulk drug.

NSC-120,949 5'-Inosinic acid, polymers, complex with 5'-cytidylic acid polymers (1:1)

Hazleton Laboratories, Inc.

Poly IC

The chronic toxicity of Poly IC was evaluated in Beagle dogs following intravenous administration three times weekly for twenty-six weeks. Dose-related signs indicate decreased medullary hematopoiesis and hepatic and renal toxicity, all of which are apparently reversible.

Reversible dose-related toxicity was reflected in elevation of serum alkaline phosphatase and scattered elevations of both transaminases. Pathologic changes included focal hepatocytic necrosis, minimal stimulation of reticulo-endothelial cells and extramedullary hematopoiesis. Episodic mild to moderate reversible uremia was apparently dose-related. Associated pathologic findings included tubular epithelial vacuolation with leukocytic infiltration, renal pelvic epithelial thickening and early glomerulosclerosis at the end of the treatment period.

The incidence of progressive mild anemia and fatty bone marrow was dose-related. Extramedullary hematopoiesis was observed in liver and spleen in treated animals, but splenic hematopoiesis was also observed in one control animal.

A few opaque bilateral strands in the vitreous humor of one high dose dog persisted from week 13 to termination (week 52). Eye changes were noted histologically in earlier studies in several dogs following daily doses although ocular abnormalities were more frequently observed in monkeys.

The toxicity associated with chronic use (26 weeks) of Poly IC is consistent with that seen following 28 daily doses. There is no evidence of any unanticipated, irreversible alteration with the possible exception of the eye changes.

NSC-121,210E Androst-5-en-17-one, 3β-hydroxy-, [p-[bis (2-chloroethyl)amino] phenyl]acetate

Mason Research Institute, Inc.

DHEA Mustard

Dehydroepiandrosterone (DHEA) is a weak androgen secreted from the adrenal glands in an amount similar to that of cortisol (15-20 mcg/day). DHEA has a potency of about one fifth that of testosterone and accounts for a major portion of the urinary 17 ketosteroids, DHEA is transformed into a sulfate ester within the adrenal gland and in pregnancy is an important precursor of estrogens formed by the fetoplacental unit. DHEA is also an estrogen precursor in the normal mature gonads of both sexes. DHEA Mustard, an analog of phenester, has been reported to cause 80% suppression of growth of the 13762 mammary adenocarcinoma in the Fisher/344 rat at 5 mg/kg/day. It has antitumor effects in rat leukemias with minimal production of endocrine side

effects. In the dog, the findings of occasional lymphopenia correlated well with histopathologic evidence of lymphoid atrophy. Leukopenia and fluctuations in differential white blood cell counts were associated with varying degrees of bone marrow atrophy. Histopathologic evaluation of uteri and mammary glands from treated bitches revealed endometrial hyperplasia and increased glandular development respectively. In male dogs, focal cellular atypia was observed in sections of testicles. Gastrointestinal toxicity was manifested overtly by anorexia, emesis, weight loss, and hypersalivation. Periodic increases in BSP retention were observed although there was no histopathologic evidence of hepatocellular destruction.

NSC-125,066 [2,4'-Bithiazole]-4-carboxylic acid, 2'-(2-aminoethyl)-, monohydrate — Mason Research Institute, Inc.

Bleomycin

Both Phleomycin (NSC-61586) and Bleomycin (NSC-125,066) are antibiotics elaborated by Streptomyces verticillus. Phleomycin was very soon found to be an extremely potent nephrotoxin. However, it was claimed that Bleomycin was largely devoid of renal toxicity. The present studies in Rhesus monkeys using the Japanese Clinical Mixture of Bleomycin have clearly established that high doses of Bleomycin can do severe damage both to the renal glomerular apparatus and to the tubules. Uremia was the sequel of this damage.

At nephrotoxic doses and below (as low as 1 mg/kg/q4d for eleven doses) characteristic Bleomycin pulmonary toxicity was detected. Two points served to distinguish this interstitial pneumonitis in the monkey from the analogous process in the dog: 1) the absence of the canine pulmonic pathogen Filaroides milksii in the monkey, the pulmonary lesions caused by parenteral Bleomycin were unequivically recognized as drug-induced. (Granulomatous effects of the simian parasite Pneumonyssus simicola posed no diagnostic problem.) 2) Whereas, Bleomycin in dogs produced pulmonary fibrosis which was largely subpleural in location, the comparable lesion in monkeys tended to extend more deeply into the parenchyma of the lung. In light of this, radiologic changes might have been anticipated in the monkey. However, chest films failed to give good evidence of pulmonary lesions in either species. At all doses discussed thus far, examples of arteritis were seen histopathologically. In different subjects, the vasculature of the bowel, heart, liver, and kidney was affected. Many of these arteritic lesions gave evidence of chronicity; there was mural hyalinization and luminal obliteration.

Miscellaneous but sometimes major inflammatory effects were also reported over the range of doses discussed so far. Thus sialadenitis and myocarditis were seen in several of the treated subjects.

The sole salient toxic effect produced by lower doses of Bleomycin (0.5-0.25 mg/kg/q4d x 11 doses) was apparent derangement of scleroprotein structure or function (dermatitis and onychoptosis). Thus, at least in the monkey, the skin and its appendages are the most sensitive targets of Bleomycin toxicity.

In summary, low doses of Bleomycin cause integumentary toxicity; intermediate doses cause integumentary, pulmonary, and vascular toxicity; while high doses damage all of the aforementioned organ systems and, in addition, are nephrotoxic when administered to monkeys.

NSC-129,943 2,6-Piperazinedione, 4,4'-propylenedi-, (±)- Yale University School of Medicine

The oral administration of ICRF 159 in tablet form to Beagle dogs resulted in greater toxic manifestations than reported previously with administration of the bulk powder in gelatin capsules. Drug-induced deaths occurred at doses of 320 mg/kg/day x 5 days and 80 mg/kg/day x 5 days with the bulk formulation and the tablet formulation, respectively. The apparent increased toxicity of the tablet formulation in test dogs was an unexpected finding. Perhaps a component of the tablet formulation which binds with ICRF 159 (i.e. magnesium) may act as a carrier, enhancing translocation across the intestinal epithelium and, thus, resulting in a higher blood concentration of the drug.

No drug-induced deaths occurred in Rhesus monkeys receiving oral doses of the bulk drug at levels from 40 to 160 mg/kg/q4d x 6 weeks. The highest nontoxic dose (HNTD) in the monkey appeared to be 80 mg/kg/q4d x 6 weeks.

Clinical and pathologic alterations observed in test monkeys were similar to those seen in the dog.

Overt signs of gastrointestinal toxicity were manifested by anorexia, adipsia, diarrhea, and resultant weight loss. These alterations correlated well with the histopathologic findings of intestinal epithelial destruction, degeneration, and leukocytic infiltration into the lamina propria.

Alterations in hematologic parameters were characterized by fluctuating increases in lymphocytes and decreases in neutrophils. An occasional reduction in platelet, WBC and RBC counts was noted. Histopathologically the bone marrow appeared to be reduced in cellularity. Mild elevations of SGOT were reported without histopathologic evidence of hepatocellular destruction.

Occasional increases in BUN were reported. No gross or microscopic renal lesions were observed.

Histopathologic evaluation of the testes revealed degenerating germ cells present in one monkey.

NSC-406,021 9H-Purine-6-thiol, 9-β-D-arabinofuranosyl- I. I. T. Research Institute

Ara-6-MP

The intravenous administration of Ara-6-MP to monkeys for fourteen consecutive days at various doses resulted in mild signs of toxicity. All experimental monkeys were sacrificed in general good condition. A male and

a female monkey were treated at each of four different dose levels. Hepatotoxicity was the major drug-induced alteration. The mild anemia observed in two of the experimental monkeys was not considered to be drug-related. Sporadic fluctuations in the white blood cell counts were noted in all monkeys; whereas, reduced platelet counts were infrequent. Liver function tests were altered frequently but were reversible. SGOT levels were elevated in six of the test animals; whereas, SGPT increased in only one of the ten experimental monkeys. Histopathologic evaluation of liver sections revealed dose-related periportal fibrosis and bile duct hyperplasia in nearly all of the test monkeys. At the two higher dose levels urinary sediment was observed in samples voided by three of six monkeys. The sediment consisted of cholesterin plates, epithelial cells, white and red blood cells, and calcium oxalate crystals. Histopathological changes in renal tissues were not evident upon necropsy.

PROTOCOL STUDIES IN PROGRESS:

NSC-4728	1,3,4-Thiadiazole, 2-amino-	South Shore Analytical & Research Institute, Inc.
NSC-68075	Thalicarpine	Arthur D. Little, Inc.
NSC-71851	9H-Purine-6-thiol, 2-amino-9-(2-deoxy-α-D erythro-pentofuranosyl)- α-TGdR	IIT Research Institute
NSC-79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- CCNU	Mason Research Institute
NSC-83265	L-Alanine, 3-(tritylthio)-	Arthur D. Little, Inc.
NSC-95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-	Mason Research Institute
NSC-109,129E	Prega-4-ene-3,20-dione, 21-hydroxy-, [p[bis(2-chloroethyl) amino]phenyl]acetate Deoxycorticosterone nitrogen	Midwest Research Institute
NSC-112,260E	Androst-4-en-3-one, 17β-hydroxy-, [p[bis(2-chloroethyl) amino]phenyl]acetate Testosterone nitrogen mustard	International Research & Development Corporation
NSC-118,994	Digylcolaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9H-purin-9-yl)-	IIT Research Institute
NSC-135,758	2,5-Piperazinedione, 3,6-bis (5-chloro-2-piperidyl)-, dihydrochloride	Battelle Memorial Institute
NSC-136,020E	Androst-4-en-3-one, 17β-hydroxy-7β-methyl-, propionate Testosterone, 7β-methyl-, propionate	Mason Research Institute

NSC-139,105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(2,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenoxy] -N,N-dimethyl-m-toluamide (1:1)	Yale University School of Medicine
NSC-143,769	Methanol extraction residue (MER) of BCG	Mason Research Institute

SPECIAL STUDIES

NSC-740	Methotrexate	University of Texas - Austin

To study the toxicity in dogs and monkeys of chronic administration of MTX (5-year study).

NSC-3053	Actinomycin D	University of Texas - Austin

To study the toxicity in dogs and monkeys of chronic administration of this drug (5-year study).

NSC-8806	Melphalan	University of Texas - Austin

To study the toxicity in dogs and monkeys of chronic administration of this drug (5-year study).

NSC-15193	2-Deoxy-D-glucose	South Shore Analytical & Research Laboratory, Inc.
NSC-132,315	Glucopyranose, 2-deoxy-2-formamide-, monohydrate, -D-	

To investigate the use of these agents as antidotes to Streptozotocin diabetes.

NSC-15200	Gallium nitrate, $Ga(NO_3)3$	Mason Research Institute

To ascertain if this metal causes ototoxicity.

NSC-19893	4-Fluorouracil	University of Texas - Austin

To study the toxicity in dogs and monkeys of chronic administration of this drug (5-year study).

NSC-23909	1-methyl-1-nitroso-urea	South Shore Analytical & Research Laboratory, Inc.
NSC-85998	Streptozotocin	
NSC-13128	Nicotinamide	

To ascertain the causative role of these agents in the production of Streptozotocin renal tumors and Streptozotocin + nicotinamide pancreatic islet tumors.

NSC-67574 Vincristine sulfate — Battelle Memorial Institute

To establish the highest non-toxic and lethal doses in Beagle dogs and Rhesus monkeys using fractions and multiples of the established clinical schedule and dose.

NSC-67574 Vincristine sulfate — University of Texas - Austin

To study the toxicity in dogs and monkeys of chronic administration of Vincristine (5-year study).

NSC-69856 Neocarzinostatin — Mason Research Institute

To compare the toxicity in dogs and monkeys of this drug as formulated by the Japanese and Bristol Laboratories.

NSC-82151 Daunomycin — International Research & Development Corporation

To establish the highest non-toxic and lethal doses in Beagle dogs and Rhesus monkeys using fractions and multiples of the established clinical schedule and dose.

NSC-85998 Streptozotocin — South Shore Analytical & Research Laboratory, Inc.

To study the effect of this agent on the reproduction capabilities of male and female mice.

NSC-106,977 L-Asparaginase, from *Erwinia Carotovora* — South Shore Analytical & Research Laboratory, Inc.

To confirm the report of delayed renal toxicity from England since our initial studies had not indicated any delayed toxicity.

NSC-107,392 Picolinaldehyde, 5-hydroxy-, Thiosemicarbazone — Mason Research Institute

To explore the possibility of preventing the violentemetic effect of this agent by concomitant administration of Δ^9 Tetrahydrocannabinol.

NSC-109,229 L-Asparaginase — Arthur D. Little, Inc.

To study, using audoradiographic techniques, the fate and distribution of L-asparaginase.

NSC-119,875 Platinum, diamminedichloro-, *cis*- — Mason Research Institute

To develop an animal model for prediction of toxicity.

NSC-119,875 Platinum, Diamminedichloro-*cis*- Mason Research Institute

To develop more sensitive parameters, than those presently used, for prediction of renal toxicity.

NSC-125,066 Bleomycin Mason Research Institute

To ascertain role of the serum metals, especially zinc, in Bleomycin pulmonary and integumentary toxicity.

NSC-125,066 Bleomycin Mason Research Institute

To explore the possibility of preventing Bleomycin pulmonary toxicity by the concomitant administration of Orgotein (erythrocuprein, a superoxide dismutase of mammalian origin - with anti inflammatory properties).

SUMMARY REPORT

LABORATORY OF TUMOR CELL BIOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 -- June 30, 1972

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, i.e., obtaining information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) cell cycle characteristics; (3) search for biochemical markers of minimal neoplastic disease; (4) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (5) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in a number of in vitro and in vivo systems. This is the ultimate goal of the laboratory.

During the past year a number of exciting findings were reported by investigators from the Laboratory:

1. DNA Polymerase of Human Cells. An extensive investigation of "DNA-dependent" and "RNA-dependent" DNA polymerase activities in normal and leukemic human cells and in some solid tumors was continued. This was prompted by: (a) the fact that the principal effect of many antitumor agents is through inhibition of DNA synthesis; (b) the finding in RNA oncogenic viruses of the first evidence for a "reverse transcriptase" (RNA-dependent DNA polymerase) which was virtually limited to the RNA tumor viruses and that this enzyme can be inhibited fairly selectively with some rifampicin derivatives; (c) the lack of any detailed information on these enzymes in fresh human specimens, particularly on their biological role; (d) the increasing evidence that RNA tumor viruses may play a role in human cancer. These viruses not only require their own DNA polymerase ("reverse transcriptase") for their replication but also require cell mitosis which in turn requires DNA replication via cellular DNA polymerases. Clearly, a study of the properties of the purified human cellular DNA polymerases, an attempt to clarify their biological functions, and a search for viral or viral-like polymerases was needed.

Only one DNA polymerase (in E. coli) was known for many years but in the last 2 years two additional DNA polymerases were discovered. Recently in this laboratory three DNA polymerases have been isolated and partially purified from human leukemic cells. One of these can catalyze DNA synthesis from an RNA template. This was the first finding of this type of enzyme in any animal cell. Although it remains to be proven whether this activity is

the same as the enzyme from RNA oncogenic viruses, the template properties and inhibition by rifampicin derivatives (first described here) gave support to this possibility. If the enzyme is associated with an RNA oncogenic virus it is theoretically possible to prevent transformation by infectious virus or "protovirus" (of Temin). The work on DNA polymerases here and elsewhere has created a great deal of excitement because of their important potential implications for the control of neoplastic disease.

Further, in the past few months, the leukemic enzyme was shown to use 70S RNA (i.e., to make a partial DNA copy). The DNA product was shown to be a specific complement of the viral RNA used as template. This has never been shown for any DNA polymerase from normal cells.

2. The reverse transcriptases from an avian tumor virus (AMV), mammalian (RLV), primate (Mason-Pfizer monkey mammary tumor virus), and from a candidate human type C RNA virus (ESP-1) were purified and characterized. We and others have learned this year that we can distinguish the purified viral enzyme from normal cellular DNA polymerases by their biochemical properties, especially the acceptance of 70S viral RNA.

3. We supplied evidence that reverse transcriptase is required for initiation of neoplastic transformation by RNA tumor viruses.

4. In collaboration with D. Gillespie, we discovered that RNA tumor viruses contain poly A and that non-oncogenic RNA viruses contain either no poly A or it is smaller. This qualitative distinction has already been put to use for extremely important practical studies on human breast cancer and leukemia.

5. tRNA Methylation. Studies of tRNA methylating activities have shown that chemically (DENA) induced hepatomas in primates have increased tRNA methylation and suggest that new tRNA methylases may appear. These changes occur prior to any histological evidence of neoplasia and are accompanied by the appearance of the serum protein, alpha fetoprotein. Similar findings (induction of methylases and alpha fetoprotein) also occur in the embryo, giving further support to the view that oncogenesis represents a form of gene derepression analogous to the fetal state.

An increase in the methylating enzymes was previously found in DNA oncogenic virus transformed cells (polyoma and SV40) compared to control cells (but not in RNA oncogenic virus transformed cells). Because of these findings the methylated bases of the tRNAs themselves were analyzed and an increase (3- to 4-fold) was found only in 3-methylcytidine in the transformed cells. An increase in methylated bases of the tRNA from human brain tumors had been reported earlier by other investigators. Stimulated by these findings a collaborative effort was undertaken with Dr. L. Levine of Brandeis University to develop antibodies against each methylated base of tRNA. This resulted in the first successful development of an immunologic approach to the detection of methylated purines and pyrimidines. The plan now is to attempt to increase the sensitivity of this method in an attempt to detect individual methylated bases in biological fluids (e.g., cerebrospinal fluid and plasma) as potential biochemical markers for the presence of neoplastic disease.

6. tRNA Profile Change in Cells Transformed by DNA Oncogenic Viruses. Several species of tRNA were evaluated by reverse phase partition column co-chromatography in a variety of biological systems. One consistent interesting finding was made: Aspartyl-tRNA was changed in DNA oncogenic virus (SV40 and polyoma) transformed cells (an extra species). No changes were found in RNA oncogenic virus transformed cells or with spontaneous neoplastic transformation. Aspartyl-tRNA in these cells was identical to those of embryonic or adult tissues or normal tissue culture cells. Further, the extra aspartyl-tRNA species in the DNA oncogenic virus transformed cells disappears when the cells were inoculated into a recipient animal to form a tumor. However, when the tumor cells were allowed to grow again in tissue culture the new species reappeared.

7. Membrane Studies. It has recently been demonstrated that the membranes of transformed cells in culture are different from control cells, e.g., agglutination by concanavalin and in some systems an altered Km for glucose uptake. Because these findings might be immediately applicable clinically, normal and leukemic (acute and chronic) leukocytes from patients in remission and relapse were examined but no significant differences were found.

8. Low Molecular Weight RNA in Normal and Neoplastic Human Cells. In collaboration with Dr. Paul Gerber, comparisons of low molecular weight RNA species (4S and 5S RNA) were made using human lymphoblasts in tissue culture derived from normal donors, patients with infectious mononucleosis, Burkitt's lymphoma, PHA transformed lymphocytes, and from EB virus transformed lymphocytes. The patterns of RNA species in PHA transformed lymphocytes, "normal" lymphoblast cell lines, and infectious mononucleosis were similar. The RNA profiles of Burkitt lymphoma and EB virus transformed cells were similar to each other but differed considerably from the others.

9. Cytogenetic Studies. Special emphasis was made on the study of 32 patients who were originally Ph^1 positive or positive-negative during a long chronic phase. These studies appear to favor the theory advocating a multiple stem cell origin of marrow cells resulting in clonal evolution of abnormal cells. Other studies included: (1) in vitro pulse labelling of H^3-thymidine as a diagnostic aid in acute leukemia; (2) use of the sex chromosome as a marker to detect leakage of fetal cells through the placenta into the maternal circulation during the course of pregnancy. There were indications that the fetal leukocyte can traverse the placenta and enter the maternal circulation at two time periods--at the end of the first trimester and immediately following delivery. (3) Hycanthone was studied on different doses in vitro human lymphocyte and in vivo on rabbit lymphocyte and fetus. The drug inhibited lymphocytes to transform by PHA at the dose of 20 γ or more. No malformation of fetus seen, but may increase resorption at high concentration.

Serial No. NCI-6117 (New)
1. Laboratory of Tumor Cell Biology
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells; On New Approaches to Cancer Chemotherapy; and On the Development of Biochemical Markers of Neoplastic Cells.

Previous Serial Number: NCI-3406

Principal Investigator: Robert C. Gallo

Other Investigators: Samuel Bobrow, Robert Gallagher, R. Graham Smith, Jacqueline Whang-Peng, T. Phillip Waalkes, Michael Paran, Marjorie Robert, Paul Gerber, Richard Adamson, French Anderson, Wallace Rowe, Natalie Teich, Doug Lowy, Dave Gillespie, and Michael Sporn

Cooperating Units: Laboratory of Viral Immunology, Division of Biologics Standards; Laboratory of Chemical Pharmacology, NCI; Section on Molecular Hematology, Molecular Disease Branch, NHLI; Laboratory of Viral Diseases, NIAID; Gerontology Research Center, NICHHD; and Carcinogenesis, NCI.

Man Years:

Total: 14 1/4
Professional: 9
Other: 5 1/4

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

   a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

   b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformations?

   c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

2. Chemical studies of oncornavirus specific RNA. Purposes:

   a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

3. Leukocyte differentiation <u>in vitro</u>. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was set up last year following the techniques of Paran and Sachs (Paran is now in our laboratory). Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Characterization of tRNA species and tRNA methylation in normal adult and embryonic cells, growing and resting tissues, and in various neoplastic cells. The rationale for this approach has been described in detail (J. Mol. Biol. 52: 195-219, 1970; Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334).

5. Developing methods for getting specific RNA species into animal cells intact and to determine their biological effects.

6. Since the methylated bases of tRNA have been shown in some laboratories to be increased in some human tumors, an approach to quantitating various methylated bases by a sensitive and specific technique may be useful in quantitating residual tumor mass. An objective this year was to develop such an assay system. Similarly, in vitro studies of membrane properties of some neoplastic cells have indicated differences from normal cells (e.g., concanavalin agglutination and glucose transport). Therefore, one objective was to determine if human leukemic cells had these properties and if advantage could be taken of this to determine if the morphologically "normal" cells of remission leukemic patients are really normal.

7. Development of potent and selective DNA polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were studied as reported (Nature 228: 927-929, 1970; Nucleic Acid-Protein Interactions and Nucleic Acid Synthesis in Viral Infection, Miami Winter Sympsoia 1971, D. W. Ribbons, J. F. Woessner, and J. Schultz, eds., North Holland Publishing Co., Amsterdam, 1971, pp. 353-376), and most recently improved techniques of purification (Molecular Studies in Viral Neoplasia, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 25th Annual Symposium on Fundamental Cancer Research, 1972, in press).

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. Product analysis of DNA synthesized by various DNA polymerases is carried out by conventional techniques including:

   a. "Nearest neighbor" analysis.

   b. Size estimate by gel electrophoresis and by glycerol gradient ultracentrifugation.

   c. Complementarity by the molecular hybridization techniques of Gillespie and Spiegelman (but Cs_2SO_4 density gradient equilibrium centrifugation rather than the filter paper approach is required).

6. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs.

7. The characterization of tRNA species (J. Mol. Biol. 52: 195-219, 1970), tRNA methylases (Cancer Res. 30: 2484-2492, 1970) have been previously described.

8. The isolation and quantitation of tRNA methylated bases from tissues were carried out by standard techniques.

9. The development of a sensitive technique for tRNA methylated bases was by an immunoassay developed by Dr. Lawrence Levine, Brandeis University (Biochemistry 10: 2009-2013, 1971).

10. Procedures for uptake of RNA into animal cells has been described (Proc. Nat. Acad. Sci. 67: 1943-1950, 1970). The demonstration of biological function of these RNA molecules added from one cell to another is evaluated in several systems.

Major Findings

1. "Reverse Transcriptase" and Other DNA Polymerases of Human Cells and Tumor Viruses.

   a. In E. coli only one DNA polymerase was known for many years. in the last few years two other DNA polymerases were discovered. Our laboratory has isolated and partially purified three DNA polymerases from normal and leukemic human lymphocytes. One polymerase can catalyze DNA synthesis from an

RNA template. This was the first finding of this type of enzyme in any animal cell. Although it remains to be proven whether this activity is the same as the enzyme from RNA oncogenic viruses, the template properties gave support to this possibility. We were, however, handicapped from the start by not having the right RNA template, viz., RNA from a known human RNA oncogenic virus, to test with the leukemic enzyme. However, during the past year we have proven that the enzyme from leukemic cells will copy the genomic 70S RNA from avian RNA tumor viruses. The DNA made is complementary specifically to the template viral RNA, i.e., true reverse transcriptase. No DNA polymerase from normal human lymphocytes has this property. In fact, to date there is no evidence in any normal cell for an enzyme which copies a single-stranded natural RNA.

b. The reverse transcriptases from three RNA tumor viruses--avian (AMV), murine (RLV), and primate (Mason-Pfizer monkey mammary tumor virus)--were purified and their characteristics determined. Their biochemical properties are very similar if not identical. All accept natural RNAs, RNA-DNA hybrids, and DNA. All are inhibited by the same rifampicin derivatives to about the same extent. This information is critical to our search for similar DNA polymerases in cells. We also purified the enzyme from a candidate human type-C RNA virus (ESP-1).

c. Poly U inhibits viral reverse transcriptase. We confirmed and extended observations made by Touminen and Kenney, however, we proved that it is not selective as they initially suggested.

d. Two hundred and three rifamycin derivatives were screened for their effects on viral and cellular DNA polymerases. Some have no effect on any cellular polymerase. A number are reasonably potent (50% inhibition below 10 μg/ml). Few show significant selectivity.

e. We supplied strongly suggestive evidence that reverse transcriptase is required for replication of oncornaviruses and for cellular transformation by these viruses via inhibitor studies (preincubating virus with various rifamycin derivatives, removing inhibitor, and assaying viral biological activity and reverse transcriptase; a direct correlation was found).

2. Cytotoxicity Studies. We found that some rifamycin derivatives have cytotoxic effects on human fresh leukocytes, some at reasonable concentrations (5-20 μg/ml). All cytotoxic compounds were potent DNA polymerase inhibitors. A few showed striking selectivity, i.e.,

lethal effects on leukemic cells at one-fifth to one-twentieth the concentrations required to kill normal proliferating (PHA stimulated) human blood lymphocytes. The mechanism of cytotoxicity is now under study.

3. Studies on Oncornavirus 70S RNA. We discovered that the 70S RNA of RNA tumor viruses contains poly A. This was found for avian (AMV), feline (FeLV and FSV), murine (MSV, RLV), and primate (MP-MTV) viruses. Duesberg has found the same in Rous sarcoma virus. Further, we find that eight non-oncogenic RNA viruses have no poly A or else its poly A is much shorter than that in the tumor viruses, i.e., we can qualitatively distinguish them and we can distinguish it from the RNA of normal cells which contains poly A (i.e., some messenger RNAs). This has led us to use our techniques as a new probe in searching human tissues for tumor virus RNA.

4. Transfer RNA Studies, Particularly in "Normal" and Virus Transformed Tissue Culture Cell Lines.

   a. Chromatographic separation and analysis of tRNA isoaccepting species. Chromatographic profile variations of certain aminoacyl-tRNAs have been noted in several types of neoplastic cells and in cells in different states of cytodifferentiation. Although this variation in cellular composition of tRNA species implies a regulatory role in cytodifferentiation, rarely has this, in fact, been experimentally related to alterations in cellular phenotype. The present studies have employed genetically and environmentally controlled cell lines to determine if tRNA differences in virus transformed cells are related to the properties of malignancy per se or more specifically to the function of transforming DNA or RNA oncogenic viruses.

      A survey of several aminoacyl-tRNAs in DNA oncogenic virus transformed cells revealed a consistent marked difference only in aspartyl-tRNA. The alteration is not related to the general properties of transformed cells. It may be related to certain virus-specific properties; studies with mutant virus are in progress to further define this. A substantial difference in lysyl-tRNA from RNA oncogenic virus transformed cells has also been found; whether this is related to select 4S RNA species in RNA oncogenic viruses remains to be determined.

   b. Transfer RNA Uptake Studies. Since macromolecules have regulatory function, it may be possible to alter cellular characteristics by pulsing macromolecules from one type of cell into another. An association of exogenously administered tRNA with recipient cells has previously been published but no definitive evidence has been

presented that the tRNA, in fact, enters the cell and gains access to the appropriate metabolic machinery where it might have some physiologic effect.

It has been demonstrated that exogenous transfer RNA can effectively penetrate cells as measured by in vivo aminoacylation. Experiments are in progress to determine if pulsed exogenous tRNA does support in vivo transfer of amino acids into cellular proteins. Attempts have been made to specifically alter cytodifferentiation by pulsing tRNA from virus transformed into control cells, with, as yet, no definitive positive results.

5. Attempted Induction of Virus from Human Leukemic Cells. Recently, several methods have been published for markedly augmenting the production of RNA oncogenic viruses from animal leukemias and sarcomas. In collaboration with Dr. Wallace Rowe's laboratory these techniques are being used in an attempt at virus induction in human leukemia cells. The possible induction of virus is being monitored by assay for reverse transcriptase activity.

   All methods with fresh leukemic cells in short term cultures have failed to disclose virus induction despite the use of an assay system able to detect very small amounts of murine leukemia virus. The results from leukemic cells which are being co-cultivated with other types of cells and from established leukemic cell lines are incomplete.

6. Use of Radiolabelled DNA Products from RNA Oncogenic Viruses to Probe for Homology Between Oncogenic Viruses and with Nucleic Acids from Human Leukemic Cells. Various aspects of these studies are being done in collaboration with various investigators in our laboratory group and no definitive results are yet available, although much of the technology has been worked out.

7. In the in vitro human leukocyte system (for studying differentiation) we have obtained and partially purified factors which enhance maturation. They are probably the same as those found by Metcalf, Robinson, Sachs, the Toronto group, and others. These are now under intensive study.

8. Previously we had examined tRNA species for all 20 amino acids in normal and leukemic cells and one major difference was found, tyrosyl-tRNA (J. Mol. Biol. 52: 195-219, 1970). The study was extended to compare normal lymphoblast cell lines with a plasma cell line derived from a patient with multiple myeloma and producing lambda chain (Bence-Jones) protein. Of four amino acids studied (aspartic acid, leucine, serine, and tyrosine) a significant difference was found only for tyrosyl-tRNA. This is the fourth system in which a finding of a tyrosyl-tRNA difference has been reported between a neoplastic and normal cell.

9. Studies of tRNA methylating activities have shown that chemically (DENA) induced hepatomas in primates have increases in tRNA methylation and

suggest that new tRNA methylases appear. These changes occur prior to any histological evidence of neoplasia and are accompanied by the appearance of the serum protein, alpha fetoprotein. These exact same findings (induction of methylases and alpha fetoprotein) also occur in the embryo, giving further support to the view that oncogenesis represents a form of gene derepression analogous to the fetal state.

10. A collaborative effort was made with Dr. L. Levine of Brandeis University to develop antibodies against each methylated base of tRNA. This was the first successful development of an immunologic approach to the detection of methylated purines and pyrimidines. The plan is to attempt to increase further the sensitivity of this method for a future approach to determining individual methylated bases in biological fluids (e.g., cerebrospinal fluid and plasma) as potential biochemical markers of tumor cells.

11. Recent developments in in vitro systems have demonstrated that the membranes of transformed cells are different from control cells, e.g., agglutination by concanavalin and in some systems an altered Km for glucose uptake. Because these findings might be immediately clinically applicable (e.g., if so-called remission leukemic patients' leukocytes showed these subtle membrane changes, they might provide a basis for quantitation of morphologically non-recognizable but nevertheless neoplastic cells), a study of normal and leukemic (acute and chronic) leukocytes was initiated two years ago. No correlations have ever been found, and the study has been terminated.

12. In collaboration with Dr. Paul Gerber, comparisons of low molecular weight RNA species (4S and 5S RNA) were made between human lymphoblasts in tissue culture derived from normal donors, from patients with infectious mononucleosis, from Burkitt's lymphoma, from PHA transformed lymphocytes, and from EB virus transformed lymphocytes. With this system the range of lymphoblasts from normal non-tissue established, normal tissue culture established, non-neoplastic but proliferative disorders, and neoplastic proliferative disorder could be compared. The patterns of the PHA transformed normal lymphocytes, "normal" lymphoblast cell lines, and infectious mononucleosis cells were similar. The RNA profiles of Burkitt lymphoma and EB virus transformed cells were similar to each other but differed considerable from the others. The origin and meaning of these differences has not been clarified. The study will not continue.

13. Uptake of low molecular wieght (4S) RNA by animal cells was shown and conditions for increasing the penetration of these molecules more than 100-fold was achieved. Uptake appears maximal in proliferating cells. Biological experiments are now in progress to study the effects of various RNA molecules from one cell on other cells.

14. In collaboration with the Laboratory of Chemical Pharmacology the mechanism of action, anti-tumor effect, resistance, and cell cycle effects of the anti-tumor agent, camptothecin, were studied. Inhibition

of DNA synthesis and prevention of mitosis probably by binding to specific proteins appears to be the prime mechanism of action. Cross resistance with other anti-tumor agents was not found.

15. Utilizing reverse transcriptase purified from avian myeloblastosis virus, we have confirmed findings of others that a DNA copy of mammalian messenger RNA can be made. In collaboration with Dr. F. Anderson of the NHLI we have made the DNA copy of messenger RNA for hemoglobin from normal reticulocytes. We will then see if we can get this DNA into the cells of patients with Thalassemia. If it enters the cell, integrates into the cell DNA and functions, it may genetically correct (in vitro) the Thalassemic defect. This will be assayed by determining if hemoglobin synthesis reverts to normal.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Honors and Awards:

Dr. Gallo

1. "A Study of Rifampicin, Rifampicin Derivatives, and Related Compounds on Normal and Leukemic Human Leukocytes and the DNA Polymerases of These Cells." Presented at the Second International Congress for Virology, Budapest, Hungary. July 1971.

2. "DNA Polymerases in Human Cells and of Human RNA Viruses." Presented at the Fifth International Symposium on Comparative Leukemia Research in Padova, Italy. September 1971.

3. "Effects of Rifampicin Derivatives on RNA- and DNA-Dependent DNA Polymerases of RNA Tumor Viruses and Human Leukemic Cells, Virus Induced Neoplastic Transformation, and on the Viability of Leukemic Cells." Presented at the Eleventh Interscience Conference on Antimicrobial Agents and Chemotherapy in Atlantic City, New Jersey. October 1971.

4. "RNA-Dependent DNA Polymerase of a Human Type C Virus and the Rationale for Antiviral (Polymerase) Inhibitors in Therapy." Presented at the Symposium on Mammary Neoplasia in Cherry Hill, New Jersey. November 1971.

5. Invited by the New York Society for the Study of Blood in New York to lecture on "RNA-Dependent and DNA-Dependent DNA Polymerases of Murine and Human Leukemia: Cellular and/or Viral Origin." November 1971.

6. O'Brian Visiting Professorship, Department of Medicine, University of Minnesota, Minneapolis, Minnesota. Invited to lecture on "RNA Tumor Viruses, Reverse Transcriptase, and Human Cancer: Rationale for Anti-Viral (Anti-Polymerase) Agents Now?'; "On the Molecular Mechanisms of Leukemogenesis: Information for New Approaches to Therapy"; "Reverse Transcriptase and Cellular DNA Polymerases: Approaches for Potential Diagnostic and Prognostic Aids and for Finding Tumor Viruses in Man." December 1971.

7. "DNA Polymerases of Human Cells and Studies of the Effects of Inhibitors on Intact Normal and Leukemic Cells." Presented at the Fourteenth Annual Meeting of the American Society of Hematology in San Francisco, California. December 1971.

8. Invited by Pfizer Inc. in Groton, Connecticut to lecture on "Reverse Transcriptase: Its Status in RNA Tumor Viruses and Potential Implications to Human Neoplasia." December 1971.

9. Invited by Smith, Kline & French in Philadelphia, Pennsylvania to lecture on "Reverse Transcriptase (RNA-Dependent DNA Polymerase) in RNA Tumor Viruses and in Cells: Current Status and Rationale for Anti-Polymerase Inhibitors." January 1972.

10. Invited by the Department of Biological Sciences, Columbia University in New York to lecture on "DNA Polymerases of Human Normal and Leukemic Cells and a Human Type C RNA Virus and Rationale for Inhibitors to Clinical Oncology." January 1972.

11. Invited by the Roche Institute of Molecular Biology in Nutley, New Jersey to lecture on "Reverse Transcriptase." January 1972.

12. Invited by Wright College in Chicago, Illinois to lecture on "Present Concepts of Oncogenesis." March 1972.

13. "The Nature and Properties of the RNA-Dependent DNA Polymerase of Human Cells." Presented at the 25th Annual Symposium on Fundamental Cancer Research: Molecular Studies in Viral Neoplasia in Houston, Texas. March 1972.

14. Bryan Priestman Memorial Lectureship of the University of New Brunswick in Fredericton, Canada. Invited to lecture on "Tumor Viruses, Theories of Viral Oncogenesis, and Relevance to Man"; "Reverse Transcriptase: The DNA Polymerase of Oncogenic RNA Viruses"; and "The Leukemic Cell." March 1972.

15. "New Concepts for Therapy of Human Leukemia." Presented at the Sixth International Congress of Cybernetic Medicine in Naples, Italy. April 1972.

16. "Reverse Transcriptase in Leukemia Viruses and Cells." Presented at the annual meeting of the Ohio Academy of Science, "Leukemia Research 1972" in Marietta, Ohio. April 1972.

17. Invited by the Department of Surgery of Georgetown University Hospital in Washington, D. C. to lecture on "Reverse Transcriptase: An Overview." May 1972.

17. "Reverse Transcriptase of RNA Tumor Viruses and Human Cancer and Comparison to Other DNA Polymerases." Presented at the Sixth International Symposium on Molecular Biology in Baltimore, Maryland. June 1972.

19. "Regulation of DNA Synthesis in Normal and Leukemic Leucocytes." Presented at the Second International Symposium on Metabolism and Membrane Permeability of Erythrocytes, Thrombocytes and Leucocytes in Vienna, Austria. June 1972.

Dr. Smith

1. "Properties of Purified DNA Polymerases from Phytohemagglutinin-Stimulated Normal Human Lymphocytes." Presented at the Fourteenth Annual Meeting of the American Society of Hematology in San Francisco, California. December 1971.

Dr. Robert

1. "Biochemical Studies in Leukemia Research." Presented at the First Symposium of the Leukemia Society of America. April 1972.

Publications:

Dr. Gallo

1. Gallo, R. C., Whang-Peng, J., and Adamson, R. H.: Studies on the antitumor activity, mechanism of action, and cell cycle effects of camptothecin. J. Natl. Cancer Inst. 46: 789-795, 1971.

2. Fujioka, S., Ting, R., and Gallo, R. C.: Transfer RNA methylases of normal cells, virus transformed cells, and tumors derived from transformed cells. Cancer Res. 31: 451-456, 1971.

3. Gallo, R. C., Yang, S. S., Smith, R. G., Herrera, F., Ting, R. C., Bobrow, S. N., Davis, C., and Fujioka, S.: RNA- and DNA-Dependent DNA polymerases of human normal and leukemic cells. In, Proceedings of the II Lepetit Colloquium: The Biology of Oncogenic Viruses,

L. G. Silvestri (ed.), (Amsterdam: North Holland Publishing Co., 1971), pp. 210-220.

4. Gallo, R. C.: Transfer RNA and transfer RNA methylation in growing and "resting" adult and embryonic tissues and in various oncogenic systems. Cancer Res. 31: 621-629, 1971.

5. Levine, L., Van Vunakis, H., and Gallo, R. C.: Serologic specificities of methylated base immune systems. Biochemistry 10: 2009-2013, 1971.

6. Gallo, R. C.: Synthesis and metabolism of DNA and DNA precursors by human normal and leukemic leukocytes: A summary of recent information. Acta Haemat. 45: 136-158, 1971.

7. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: Methylation of transfer RNA by monkey liver and hepatoma induced by N-nitrosodiethylamine. Arch. Int. Pharmacodyn. 191: 189-195, 1971.

8. Gallo, R. C., and Whang-Peng, J.: Enhanced transformation of human immunocompetent cells by dibutyryl adenosine cyclic 3',5'-monophosphate. J. Natl. Cancer Inst. 47: 91-94, 1971.

9. Fujioka, S., and Gallo, R. C.: Aminoacyl transfer RNA profiles in human myeloma cells. Blood 38: 246-252, 1971.

10. Gallo, R. C., Sarin, P. S. Allen, P. T., Newton, W. A., Priori, E. S., Bowen, J. M., and Dmochowski, L.: Reverse transcriptase in type C virus particles of human origin. Nature 232: 140-142, 1971.

11. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: Transfer RNA methylase activity in normal monkey liver and in carcinogen induced hepatoma. Cancer Res. 31: 1069-1973, 1971.

12. Gallo, R. C., and Whang-Peng, J.: Observations on the regulatory effects of the transfer RNA minor base, N^6-Δ^2-isopentenyladenosine, on human lymphocytes. In, Beers, R. F., and Braun, W. (eds.), Biological Effects of Polynucleotides (New York: Springer-Verlag, 1971), pp. 303-334.

13. Gallo, R. C., Yang, S. S., Smith, R. G., Herrera, F., Ting, R. C., and Fujioka, S.: Some observations on DNA polymerases of human normal and leukemic cells. In, Nucleic Acid-Protein Interactions and Nucleic Acid Synthesis in Viral Infection, Miami Winter Symposia 1971, Ribbons, D. W., Woessner, J. F., and Schultz, J. (eds.), (Amsterdam: North Holland Publishing Co., 1971), pp. 353-376.

14. Gallo, R. C.: Reverse transcriptase, the DNA polymerase of oncogenic RNA viruses. Nature 234: 194-198, 1971.

15. Gallo, R. C., and Adamson, R. H.: Asparagine, asparaginase and t-RNA. Colloques Internationaux C.N.R.S., No. 197, pp. 121-132, 1971.

16. Herrera, F., Adamson, R. H., and Gallo, R. C.: Transfer RNA as an informative molecule and its uptake by normal and leukemic human and murine leukocytes. In, Informative Molecules in Biological Systems, Ledoux, L. G. H. (ed.), (Amsterdam: North Holland Publishing Co., 1971), pp. 377-397.

17. Gallo, R. C.: RNA-dependent DNA polymerase in viruses and cells: Views on the current state. Blood 39: 117-137, 1972.

18. Fujioka, S., O'Hopp, S., Gerber, P., and Gallo, R. C.: Comparison of chromatographic patterns of low molecular weight RNA from Burkitt lymphoma, infectious mononucleosis, EB-virus transformed, and normal human lymphoblasts. Blood, in press.

19. Yang, S., Herrera, F., Smith, R. G., Reitz, M., Lancini, G., Ting, R., and Gallo, R.: Rifamycin antibiotics: Inhibitors of Rauscher (Murine) leukemia virus reverse transcriptase and of purified DNA polymerases from human normal and leukemic lymphoblasts. J. Natl. Cancer Inst., in press.

20. Gallo, R. C., Smith, R. G., Whang-Peng, J., Ting, R. C. Y., Yang, S. S., and Abrell, J. W.: RNA tumor viruses, DNA polymerases, and oncogenesis: Some selective effects of rifampicin derivatives. Medicine, in press.

21. Wu, A. M., Ting, R. C., Yang, S. S., Gallo, R. C., and Paran, M.: RNA tumor virus and reverse transcriptase: I. Biochemical studies on the ESP-1 particles. II. Role of the reverse transcriptase in murine RNA tumor virus. In, Proceedings of the Vth International Symposium on Comparative Leukemia Research (Amsterdam: North Holland Publishing Co.), in press.

22. Gallo, R. C., Abrell, J. W., Robert, M. S., Yang, S. S., and Smith, R. G.: Reverse transcriptase from Mason-Pfizer monkey tumor virus, avian myeloblastosis virus, and Rauscher leukemia and its response to rifampicin derivatives. J. Natl. Cancer Inst., in press.

23. Waalkes, T. P., and Gallo, R. C.: Clinical aspects and treatment of acute leukemia. In, Textbook of Internal Medicine, H. H. Fudenberg (ed.), (New York: Grune & Stratton), in press.

24. Torelli, U., and Gallo, R. C.: The Leukemic Cell. In, Textbook of Internal Medicine, H. H. Fudenberg (ed.), (New York: Grune & Stratton), in press.

25. Torelli, U., and Gallo, R. C.: Etiology of Leukemia. In, Textbook of Internal Medicine, H. H. Fudenberg (ed.), (New York: Grune & Stratton), in press.

26. Gallo, R. C.: Viral oncogenesis, reverse transcriptase, and human malignancy. In Textbook of Internal Medicine, H. H. Fudenberg (ed.), (New York: Grune & Stratton), in press.

27. Ting, R. C., Yang, S. S., and Gallo, R. C.: Reverse transcriptase, RNA tumour virus transformation and derivatives of rifamycin SV. Nature, in press.

28. Schrecker, A. W., Sporn, M. B., and Gallo, R. C.: Inhibition of RNA-dependent DNA polymerase by thymidylate derivatives. Cancer Res., in press.

29. Gillespie, D., Marshall, S., and Gallo, R.: RNA of RNA tumour viruses contains poly A. Nature, in press.

30. Gallo, R. C., Sarin, P. S., Sarngadharan, M. G., Reitz, M. S. Smith, R. G., and Bobrow, S. N.: Viral 70S RNA directed DNA synthesis with a purified DNA polymerase from human acute leukemic cells. In, Molecular Studies in Viral Neoplasia (The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, 25th Annual Symposium on Fundamental Cancer Research, 1972), in press.

Dr. Smith

1. Smith, R. G., Whang-Peng, J., Gallo, R. C., and Ting, R, C.: Selective toxicity of rifamycin derivatives for leukaemic human leukocytes. Nature, in press.

Dr. Robert

1. Robert, M., Smith, R. G., Sarin, P., Abrell, J. W., and Gallo, R. C.: Comparison of viral and cellular DNA polymerase activities with synthetic and natural RNA templates. Science, in press.

Serial No. NCI-6118 (New)
1. Laboratory of Tumor Cell Biology
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: A) Mechanism of Action of Select Tumor Agents; B) Biochemical Markers of Malignancy; C) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents.

Previous Serial Number: NCI-3417

Principal Investigators: T. Phillip Waalkes, Robert C. Gallo, Richard Adamson.

Other Investigators: R. Graham Smith and Y. Hirshaut.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI; Sloan-Kettering Institute, New York.

Man Years:

Total: 3
Professional: 2
Other: 1

Project Description:

Objectives

A) To obtain information regarding the biochemical and pharmacologic mechanisms whereby chemotherapeutic agents, known to be at least partially effective against various malignancies in man, appear to act. It is considered that information of this type will be helpful not only in the selection of possible analogs, the synthesis of specific compounds, and a rational approach to combination therapy, but also in the understanding of the basic biochemical processes of malignancy and malignant cell growth. The project completed during the year involved the study of tRNA methylation in specific neoplastic systems and the influence of selected chemotherapeutic agents. Various reports have confirmed the finding that hypermethylation of tRNA is a characteristic of the tumor cell. Although the function served by the addition of methyl groups to the nucleic acid bases of tRNA is not clear, the important role of tRNA in protein synthesis lends further emphasis to the possibilities.

B) Because of the need for sensitive methods for determining not only the presence of malignant cells but also their number in any patient with

cancer, a Biologic Markers Program has been initiated. The Program objective is to develop specific and sensitive techniques to detect and to measure biochemical or biological materials which are quantitative indicators of the cancer cell. Such markers are important in the evaluation of response to therapy, in determining the course of disease, and in estimating the amount of residual tumor.

C) Specific enzyme reactions involving RNA and DNA are being studied from a variety of approaches (see Dr. R. C. Gallo's annual report). In particular, RNA dependent DNA polymerase and DNA dependent DNA polymerase in several different tissues and types of cells are being evaluated as to their relationship to tumor cell growth and proliferation. Where unique features exist or where specific differences are found which may be therapeutically exploited, careful evaluation will be made to determine the best methods and means of approach.

Methods Employed

A) The L1210 system both in vitro (tissue culture) and in vivo was used to evaluate the effects of specific chemotherapeutic agents on the methylation of tRNA. The agents studied included methotrexate, vincristine, BCNU, poly IC, 6-MP, and MIH. In vitro, methotrexate had a definite inhibitory effect on methylation at 24 to 48 hours. None of the other compounds showed a similar effect. Further studies indicated that methotrexate also caused a profound inhibition on protein synthesis in the same system under similar conditions. This effect may well be the explanation for the changes found in the decreased methylation of tRNA.

B) A plan for a Biologic Markers Program is being developed. As promising leads and potential markers are properly identified, they will be studied by use of the most appropriate methods of purification and analysis. A candidate may be any type of biologic material, protein or non-protein. Studies will concentrate both on practical methods of assay and on techniques which can be utilized for following the course of a patient's disease and for quantitative assessment of the amount of tumor present. Assays will involve body fluids because of their ready accessibility.

An "in house" program has been initiated and a more extensive supplementary contract phase envisioned.

Initial efforts to develop biologic markers have concentrated on the methylated nucleic acid derivatives in blood and urine of cancer patients because (1) tRNA methylase activity is increased in tumor tissue compared to normal; (2) elevated levels of specific methylated nucleic acid bases have been reported in animal tumors and in human brain tumors; (3) increased amounts of specific methylated nucleic acid derivatives have been reported in the urine from animals with tumors and in the urine from acute leukemia patients; and (4) new, sensitive, and practical methods for separation and assay have been developed. Recently, polyamines have also been reported in the literature as found in increased amounts in the

urine of cancer patients. Initial studies have been started to evaluate these important compounds as biologic markers.

Three contracts have been funded and are in progress:

1) Liquid-chromatographic techniques--Oak Ridge National Laboratories (Dr. Charles Scott)--using high resolution, high pressure, ion exchange columns with a variety of different analytical detectors. Studies in blood and urine--methylated nucleic acid bases and then the polyamines.

2) Gas-liquid chromatography--University of Missouri (Dr. Charles Gehrke)--Studies in urine and blood--methylated nucleic acid derivatives and the polyamines.

3) Radioactive immunoassay as a sensitive analytical technique--Brandeis University (Dr. Irving Levine)--specific methylated guanines in blood, spinal fluid, and urine.

These projects are currently developing proper techniques and experimental conditions using standard compounds. Preliminary studies with urines and blood samples from patients with cancer are being made.

Reports have shown that specific malignancies in humans (i.e., hepatomas, etc.) frequently are associated with the presence of an α-fetoprotein. A similar situation exists in monkeys harboring a hepatoma induced by the simple chemical, N-nitroso diethylamine. Of interest, the α-fetoprotein appears prior to any histologic evidence of a liver tumor. Consequently, the α-fetoprotein is indicative of a premalignant state. Of further interest, studies of the methylation of tRNA show hypermethylation compared to normal liver, not only with extracts of hepatoma tissue but also with "normal" liver of monkeys with a positive test for α-fetoprotein. Further exploration of these biochemical differences is contemplated. Studies of this monkey tumor system to determine if α-fetoprotein can be used as a quantitative marker for malignancy are planned. Extension of similar techniques in the study of human hepatomas, etc., will follow.

In addition, a natural inhibitor of tRNA methylase activity is present in normal liver extracts but absent in fetal or newborn liver and hepatoma. Further studies are considered in an effort to determine the nature and activity of these inhibitors and to evaluate their presence or absence in body fluids or tissues of patients with malignancy as a further means of developing biologic markers.

C) (See Dr. R. C. Gallo's annual report). Participated in studies which involved specific tissues and cells including RNA viruses (Rauscher murine leukemia virus, Moloney sarcoma virus, and Feline leukemia virus), human lymphocytes grown in culture (#1788), PHA stimulated normal lymphocytes, and acute leukemia WBC. Enzymes were purified, defined, and characterized.

Antibody and inhibitor studies were carried out on RNA dependent DNA polymerase and on DNA dependent DNA polymerase. Specific viral (Moloney sarcoma and Feline leukemia) reverse transcriptase (RT) has been purified and has been injected into rabbits (Dr. Hirshaut) in order to produce anti-RT antibodies. Efforts to detect RT in plasma and serum of patients with acute leukemia and other malignancies by various biochemical enzymatic techniques have been unsuccessful, although is some of the samples, by preliminary assays, a DNA polymerase appears to be present. Because of in vivo tumor inhibition by certain chemotherapeutic agents which are also anti-viral compounds, Ca-256 cells have been studied for polymerase activity and to detect the possible existence of a RT. DNA polymerases have been found and tentative assessment indicates the presence of a RT. These enzymes are being purified, characterized, and studied using specific inhibitors and chemotherapeutic anti-tumor compounds.

Honors and Awards

None

Publications

1. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., And Gallo, R. C.: Methylation of tRNA by monkey liver and hepatoma induced by N-nitrosodiethylamine. Arch. Int. Pharmadocynamie et Therap. 191: 189-195, 1971.

2. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: tRNA methylase activity in normal monkey liver and carcinogen induced hepatoma. Cancer Res. 31: 1069-1073, 1971.

3. Waalkes, T. P., Adamson, R. H., Riddick, D., and Gallo, R. C.: Evaluation of anti-tumor agents on tRNA methylase activity of leukemia L1210. Arch. Int. Pharmadocynamie et Therap., in press.

Serial No. NCI-6119 (New)
1. Laboratory of Tumor Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cytogenetic Studies in Patients with Neoplastic, Hematologic, and Congenital Diseases; The Effect of Drugs and Viral Transformation upon Chromosomes of Cells Either In Vivo or In Vitro; Maturation Factors in Myeloid Series In Vitro; Transplacental Passage of Fetal Leukocytes into Maternal Blood; Isolation of Megakaryocyte.

Previous Serial Number: NCI-3414

Principal Investigator: Jacqueline Whang-Peng

Other Investigators: Seymour Perry, Paul P. Carbone, Edward S. Henderson, George Canellos, Robert G. Graw, Jr., Harvey Gralnick, Richard Adamson, Sarah Steward, Sanford Leikin, Turid Knutsen, and Elaine Lee.

Cooperating Units: Clinical Oncology, NCI; Laboratory of Chemical Pharmacology, NCI; Clinical Pathology Department, CC; Georgetown University, Pathology Department; Children's Hospital of D. C.

Man Years:

Total: 6
Professional: 4
Other: 2

Project Description:

Objectives

1. Cytogenetic studies in patients with leukemia or other malignancies.
2. Study of patients with CML in blastic crisis.
3. Use of in vitro pulse labelling of H^3-thymidine as a tool for diagnosing the difficult diagnostic patient with acute leukemia.
4. Study of the blood from pregnant women to evaluate the possibility of migration of fetal white cells through the placenta.
5. Evaluation of drugs (hycanthone) in vitro which affect DNA, RNA, and protein and chromosomal aberration in human lymphocytes and fetus toxicity in rabbits.
6. Further evaluation of the maturation factor in both liquid and agar systems.
7. Isolation of megakaryocyte.

Methods Employed

Standard cytogenetic techniques were used except as specified.

Major Findings

1. In Vitro Pulse Labelling of H^3-Thymidine as a Diagnostic Aid in Acute Leukemia. We have taken peripheral blood from 31 patients with various forms of acute leukemia. In leukemias characterized by granulocytic proliferation for labelling index was 2-30%, and the labelled granulocytes ranged from 10-80%. The acute leukemias of lymphoid origin have a very low labelling index with no maturing labelling granulocytes. In three patients originally diagnosed as acute lymphocytic leukemia, the H^3-thymidine labelling was characteristic of a granulocytic leukemia. Further investigation revealed that one patient had a Ph^1 chromosome. Another patient, on review of the bone marrow and peripheral blood, was considered to be a granulocytic leukemic, and the third patient could not be restudied due to an unresponsive course while on chemotherapy.

2. The sex chromosome was used as a marker to detect leakage of fetal cells through the placenta into the maternal circulation during the course of pregnancy. PHA-stimulated peripheral blood cultures from healthy pregnant women were used for this study. Twelve of the 14 women with male cells gave birth to male babies. In one instance, follow-up studies after delivery of a male infant showed male cells in maternal blood up to 6 months. Our results seem to indicate that the fetal leukocyte can traverse the placenta and enter the maternal circulation at two time periods. One occurs at the end of the first trimester, and the other immediately following delivery. The passage of these cells may be important in the immunologic phenomena of parity related to paternally derived transplantation antigen.

3. Hycanthone. We studied hycanthone on different doses in vitro human lymphocyte and in vivo on rabbit lymphocyte and fetus. In vitro--hycanthone begins to effect chromosomes at dose 10 γ; chromosomes become elongated and sticky. Inhibited one lymphocyte to transform by PHA at the dose of 20 γ or more, if the drug was added at 0^o. If the drug was added at 44^o, up to 30% of minor and major aberrations at the dose of 20 γ. No malformation of fetus seen, but may increase resorption at high concentration.

4. Special emphasis was made on the study of 32 patients who were originally Ph^1 positive or positive-negative during a long chronic phase, but who subsequently exhibited their chromosomal abnormalities prior to or during blastic crisis. Hyperdiploidy was the most commonly observed form of aneuploidy with a high incidence of G group involvement. Seven patients had marker chromosomes. In all patients the disease terminated in blastic crisis; the median survival was 3 months. These studies appear to favor the theory advocating a multiple stem cell origin of marrow cells resulting in clonal evolution of abnormal cells.

Honors and Awards

Arthur Flemming Award, 1971.

Publications

1. Whang-Peng, J., Perry, S., Knutsen, T. A., and Gart, J. J.: Cell cycle characteristics, maturation, and phagocytosis in vitro of blast cells from patients with chronic myelocytic leukemia. Blood 38: 153-161, 1971.

2. Canellos, G. P., DeVita, V. T., Whang-Peng, J., and Carbone, P. P.: Hematologic and cytogenetic remission of blastic transformation in chronic granulocytic leukemia. Blood 38: 671-679, 1971.

SUMMARY REPORT

ASSOCIATE SCIENTIFIC DIRECTOR FOR CLINICAL ONCOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1971 - June 30, 1972

The Clinical Oncology Area has four major program goals identified in the Linear Array of the Chemotherapy Program, NCI. These are I. Initial clinical trials of antitumor agents (phase I studies, II. Screening for antitumor activity in man of new antineoplastic (phase II) agents, III. Eradicative trials of intensive combination chemotherapy (phase III) in specific diseases, and IV. Supportive care including the protected environment, platelet and white cell transfusions, and marrow transplantation. There are four clinical units actively involved in this research effort. These are the NCI-VA Medical Oncology Branch based at the Washington Veterans Administration Hospital under Dr. Oleg Selawry, the Medicine Branch (formerly the Solid Tumor Service) under Dr. V. T. DeVita, the Hematology and Supportive Care Branch (formerly the Acute Leukemia Service) under Dr. E. S. Henderson, and the NCI-Uganda Cancer Institute at Makerere University under Dr. J. L. Ziegler.

Each unit is oriented around the study of specific neoplastic diseases with supporting laboratories for cell kinetics, pharmacology, and immunology. More recently a central operations office has been established to facilitate the data flow and analysis.

A major expansion of our intramural program has occurred by the inclusion of collaborative research programs with clinical research contract efforts at several outside institutions, such as the Mayo Clinic, Rochester, Minnesota; the M.D. Anderson Hospital, Houston, Texas; Roswell Park, Buffalo, New York; and Memorial Hospital, New York, New York. Clinical trials are being supported by the Chemotherapy area to increase our ability to investigate new agents in specific disease conditions. In addition, collaborative efforts have been re-established with the Eastern Cooperative Oncology Group and expanded with the Acute Leukemia Group B by active participation of our staff members in their leadership or protocol chairmen. Finally, leadership and direction is provided in large disease oriented programs of the NCI through the Lung Working Party and the Breast Cancer Task Force by some of our senior people who have major roles in these organizations. Thus, the disease types under active investigation in the Clinical Oncology Area include lung cancer, malignant lymphomas, breast and ovarian cancer, the acute leukemias,

Burkitt's tumor, and hepatocellular carcinoma. In all these disease types, investigation involving the evaluation and workup, staging, initial and subsequent therapies, and supportive care are actively underway. Brief summaries of the highlights of the research program are described below.

I. Initial Clinical Trials:

In Fiscal Years 1971 and 1972, four Phase I drug studies were completed. These included bleomycin (NSC 125066), Poly I-C (NSC 120949), imidazole carboxamide dimethyltriazeno bis-chloroethyl (NSC 82196), and camptothecin (NSC 100880). Three new drugs were begun on Phase I clinical trials. These are methyl CCNU (NSC 95441), isophosphamide (NSC 109742), ICRF-159 [bis (3-5 dioxopiperazine)]. These drugs have been made available to our own disease-oriented program as well as to other groups such as the Mayo Clinic, the Eastern Cooperative Oncology and Leukemia Group B Groups. New trials are planned with gallium nitrate (NSC 15200), thalicarpine (NSC 68075), and a new podophyllotoxin derivative (4'-demethyl-epipodophyllotoxin-β-D-ethylidine glucoside (NSC 141540).

Evaluation of new agents involves the understanding of the pharmacologic disposition. As part of the initial clinical trials, pharmacologic studies of camptothecin, cyclophosphamide, bleomycin, isophosphamide, methyl CCNU, and ICRF-159 have been done, tying together the clinical effects and pharmacologic disposition studies and representing an ideal way to evaluate new agents in man.

II. Screening for Antitumor Activity in Man (Phase II):

The concept of the Phase II clinical trials has changed drastically from one of concentrating on testing the agent in many disease types to that of careful investigation with appropriate controls in specific disease sites. Moreover, an intensive search is being developed to find effective new agents capable of inhibiting the RNA dependent DNA polymerase or non-myelosuppressive agents for combination studies. Much of the clinical effort in the Phase II area has been shifted to extramural programs for breast, ovarian, and lung cancer, through the use of clinical contracts or collaboration with cooperative groups. This year a major finding has been uncovered in a study under the chairmanship of Dr. O. Selawry and Dr. H. Hansen of an ALGB protocol, of the superiority of CCNU over BCNU. Clinical trials of CCNU have also been done in breast and colon cancer, indicating some degree of activity but not clearly superior to BCNU.

In acute leukemia patients, new agents have been sought that might inhibit virus infection or replication. The first one tested was poly I poly C that was clearly not effective because of rapid lysis in vivo. Rifamycin-SV was put into clinical trials as a possible inhibitor of the reverse transcriptase an enzyme shown to be present in some human leukemic cells. Preliminary results with this agent demonstrate no antileukemic effect per se at levels that clearly inhibit the enzyme.

New antineoplastic agents coming out of phase I trials that cross the blood

brain barrier or show a high degree of activity in L1210 are routinely tested in resistant patients with acute leukemia. Activity has been seen for epipodophyllotoxin,4'-de-methyl-9-(4,6-O-2-thenylidene-β-D-glucopyranoside (NSC 122,819), parenteral podophyllotoxin derivative. Current clinical trials involve high dose cytoxan and methyl CCNU.

While immunotherapy has clearly been shown to affect tumor growth in animals, clinical effectiveness has not been demonstrated in a properly controlled trial. One of the reasons for this inability to demonstrate activity is related to the lack of proper in vitro parameters to follow. At the NCI and in Africa, clinical exploratory studies of immunotherapy have been done in patients with acute leukemia, Burkitt's, and melanoma in a controlled fashion and closely tied into laboratory studies of immune reactivity. These studies have employed BCG in vivo and parameters of in vitro response of lymphocytes to tumor cells or skin testing with membranes. To date the laboratory parameters have failed to indicate any evidence of sensitization by BCG of the patient or his lymphocytes to autologous tumor. The clinical effectiveness of BCG with or without cells in prolonging remissions or disease-free intervals is still too early to evaluate. All the immune manipulations are occurring in clinical situations where residual tumor is minimal, i.e., acute leukemia and Burkitt's tumor in remission or melanoma patients post primary surgery.

Graft-versus-host disease has been reported to successfully enhance the cell kill of chemotherapy in animals. A clinical protocol is underway to investigate the role of marrow transplantation in patients resistant to conventional chemotherapy utilizing HL-A identical siblings. Eight of nine patients were engrafted successfully with chimerism achieved for up to 600 days. GVH occurred in four patients resulting in fatal sequelae in two patients. The current investigation involves laboratory efforts in dogs to abate the GVH reactions and possible use of autologous marrow rescue.

III. Eraticative Trials (Phase III):

1. Hodgkin's Disease

Five year follow-up is now available in the original combination chemotherapy of Hodgkin's disease. Sixty percent of the entire group is alive after five years. Of the complete responders 77% are alive after five years and 41% are continuously free of disease without therapy after the first six months of treatment. Since 1967, complete responders have been allocated to three different regimens: (1) no further therapy, (2) intermittent treatment with drug combination for an additional six to 15 months, and (3) intermittent treatment with the new agent BCNU. Results indicate no current advantage for any regimen.

Other findings of interest in Hodgkin's disease have included the demonstration that (1) patients previously treated with radiotherapy who recur can be successfully treated with the multiple drug combination; (2) that the patients with Stage IIIB disease treated with combination chemotherapy have

a better survival and response than those treated with total nodal radiotherapy; (3) that BCNU has activity in patients who are resistant to alkylating agents. We have also shown that Hodgkin's disease is responsive to bleomycin given in a five-day course.

2. Lymphocytic Lymphoma

Since 1967 the randomized control clinical trial of optimal chemotherapy versus optimal radiotherapy in patients with Stage III and IV disease has been underway. Drug approach has involved the use of a three-drug combination of cytoxan, vincristine and prednisone. Thirty-five patients have been evaluated, 57% have achieved complete remission; the median survival and duration of remission have not yet been reached.

3. Burkitt's Tumor

One hundred and twenty-two patients with Burkitt's tumor were admitted to the Lymphoma Treatment Center in the past 4-1/2 years; the first 57 were treated initially with one or six doses of cyclophosphamide in randomized trial. This trial indicated that in patients with Stage I and II disease a single dose of cyclophosphamide was sufficient to induce durable and long-term complete remissions (75% at 3 years). Patients with Stage III disease responded equally well to intensive chemotherapy with six courses or one course; however, patients with Stage IV disease were refractory to cyclophosphamide (60% survival at 3 years). Subsequently, a combination of cytoxan, vincristine, methotrexate and cytosine arabinoside (31% survival at 3 years), was employed; results indicate to date that this is a highly effective way of inducing complete remissions; however, patients have relapsed on this regimen that is not better than cyclophosphamide alone. Central nervous system involvement continues to be a major therapeutic problem in Burkitt's lymphoma; several clinical trials have been undertaken including prophylactic intrathecal chemotherapy which has not been shown to be completely effective. In addition, studies of methotrexate or cytosine arabinoside given daily for 10 days also result in CNS recurrences. The present protocol involves the use of a combination of methotrexate and cytosine intrathecally with hydroxyurea orally. A second randomization includes the use of cyclohexylnitrosourea, a lipid soluble antitumor agent known to penetrate the blood brain barrier.

4. Lung Cancer

Intensive combination chemotherapy, assisted by radiotherapy where indicated resulted in unexpected high response rates and survival in patients with small cell, adeno, and large cell anaplastic bronchogenic carcinoma, while results in epidermoid carcinoma were not improved in a total of 44 patients. Concepts gained in the first study were advanced to a comparative clinical trials in cooperation with other clinical centers. Preliminary data imply marked superiority of a three-drug combination of CCNU + cytoxan - methotrexate over a two-drug combination (cytoxan + methotrexate; with or without radiotherapy) in patients with small cell carcinoma.

A multidisciplinary Working Party for Therapy of Lung Cancer was established in 1971. A comprehensive master-program was developed. Initiation of the first treatment protocol is imminent. Basic and clinical concepts will be advanced in an international workshop on October 16-20, 1972.

5. Acute Leukemia

Several major protocols were completed during 1971; "New POMP" for previously untreated children with acute lymphoblastic leukemia (ALL). The 66 children entering this protocol received four-drug combination of POMP at approximately weekly intervals to the point of complete remission followed by a similar schedule of combination drug administration for either six or 12 months thereafter. At the six month juncture of each arm of the maintenance protocol a single maximally tolerated dose of BCNU was administered. Fifty-eight patients achieved complete remission. Fourteen patients relapsed during the first six months of intensive chemotherapy. Twenty-one patients received no further therapy after six months and of those all but one has relapsed. Of 23 patients receiving six additional months of therapy eight remain in complete remission. The overall median duration of remission was 12 months. The reinduction rate was 60%. In the adult ALL protocol (ALGB 6812) also completed in 1971, POMP was compared to the three-drug combination of vincristine, prednisone, and daunorubicin. The induction rates for both regimens was 50%. Patients induced with POMP were maintained with intermittent POMP courses while patients induced with VDP received intermittent courses followed by intermittent injections of methotrexate with periodic reinduction with the inducer agents. The median duration of remission was identical for the two maintenance programs. The protocol was revised to include L-asparaginase during remission induction consolidation and maintenance with this revised protocol the VDP plus asparaginase arm exhibited superior remission induction and remission maintenance capabilities.

The first run acute myelocytic leukemia protocol also has reached completion. This protocol compared two separate five-drug combinations: on the one hand prednisone, vincristine, methotrexate, 6-mercaptopurine, and asparaginase, on the other prednisolone, vincristine, cytarabine, daunorubicin and asparaginase. Remission rates for the two regimens were identical. The combined remission rates for adult patients (greater than 15 years of age) was 37.5% with a median duration of remission of 257 days. For patients 15 years of age and under the remission rate was 91% with an 11+ month median duration of remission, and a survival median of 18+ months.

Current first line protocols for acute lymphocytic leukemia include remission induction with vincristine, prednisone and asparaginase, intensive multi-drug consolidation, central nervous system, x-ray plus intrathecal methotrexate during the consolidation period, and multiple drug therapy during remission. The childhood protocol is modified to randomly allocate patients once every six months to two-month cycles of intermittent methotrexate or two-month cycles of immunotherapy with BCG plus live allogeneic leukemia blast cells. In acute myelocytic leukemia the efficacy of twice daily cytarabine plus 6-thioguanine is under investigation, and preliminary evidence supports the high remission rate obtained with this technique by Clarkson at Sloan Kettering.

The remission maintenance phase of this protocol compares cytosine arabinoside alone with cytosine arabinoside in combination with hydroxyurea, an association shown to be synergistic in animal studies. BCNU is intercalated into this regimen with a schedule predicted by animal data to reduce the toxicity of the combination.

Combinations of asparaginase plus azaserine, asparaginase plus azotomycin, and asparaginase plus actinomycin D are being compared to asparaginase alone in remission induction of acute lymphocytic leukemia. There is no evidence to date that these combinations were superior to asparaginase alone in remission induction of these patients.

Phase II trials are being conducted with the experimental agents poly I-poly C, bleomycin, Rifamycin SV, and VM 26, and with a high-dose, intermittent schedule of daunorubicin. Activity against acute myelocytic leukemia was noted with VM 26. Poly IC, bleomycin and Rifamycin SV have thus far not shown antileukemic activity in the doses administered. High dose intermittent daunorubicin appeared inferior. Daunorubicin administered at a standard dose and schedule.

6. Breast Cancer

The adjuvant chemotherapy protocol using phenylalanine mustard has been activated and is in existence now. It has been adopted by the Eastern Cooperative Oncology Group and the National Surgical Breast Program. Whereas we have not been able to get enough cases at the Institute, the study should now be rapidly done by these two large cooperating institutions. In addition, a multiple drug combination chemotherapy protocol has been started utilizing the principles learned from the previous Hodgkin's treatment. These have included the use of combination chemotherapy intermittently allowing for host and marrow recovery between treatments. The study to date has indicated that combination of 5-fluorouracil, methotrexate, prednisone and cyclophosphamide is tolerable when given in 14-day schedules with an intermittent and treatment period of two days. This study has been expanded and included in the Eastern Cooperative Oncology Group. So far, about 50% of the patients have achieved excellent clinical remissions.

7. Ovarian Cancer

This disease is one of the three common malignancies in women. Preliminary efforts to establish an intensive treatment protocol have begun using high dose cyclophosphamide. Along with these studies, data will be incorporated from Phase II studies done on contract to produce an effective combination.

IV. Supportive Care:

1. Laminar Air Flow - Protected Environment

The Hematology and Supportive Care Branch has as one of its main goals the development of protected environments for patients receiving intensive chemotherapy. Portable laminar air flow units have been expanded from one in

October 1969, to six, including two at the Washington Veterans Administration Hospital. These units combine laminar flow and high efficiency filtration and provide a more convenient isolation area for patients. The numbers of organisms in the air and on the environmental surface have been markedly decreased. Patients are randomly allocated to Group I, who receive antibacterial baths, sprays, topical antisepsis, and oral antibiosis and a low bacterial diet and are confined to the Laminar Air Flow Isolator. Group II patients are given the same oral, nonabsorbable antibiotics as patients in Regimen I but otherwise treated conventionally; in Group III the patients are treated in the conventional manner except with no antimicrobial prophylaxis.

Recent evaluation of these techniques has indicated a dramatic and statistically significant decrease in the severe pseudomonas infections, 27% of Group III patients to 6% and 0% of Group II and II, respectively. Of great importance was the fact that no infectious deaths occurred during induction chemotherapy in Group I patients out of 16 consecutive trials. Twenty-two percent of Group I and 27% of Group III patients died of severe infection as the primary cause of mortality. Most infectious deaths in Group III were caused by pseudomonas while the deaths in Group II occurred with a variety of other organisms other than pseudomonas. It was also important to note that the oral antibiotics did not increase the incidence of fungal infections. Infectious complications were less frequent at all granulocyte levels; the patients in Group I and II experiencing one-third as many days with severe infection as patients in Group III at the same granulocyte level. Moreover, the time to onset of first infection was prolonged significantly for Group I and II. This would mean that the oral antibiotics and the isolator allow sufficient time to administer adequate chemotherapy. While the results of induction chemotherapy indicated no difference among the three groups, there was a markedly better survival for the patients in the protected environment. Twelve out of 15 patients survived for more than 100 days, 25 of 35 in Group II, and 13 of 24 in Group III.

2. Leukocyte Transfusions

In a prospective study normal granulocytes obtained through either continuous flow centrifugation (IBM-NCI cell separator) or by continuous filtration leukaphersis (technique of Djerassi) were given to 39 recipients with acute leukemia, granulocytopenia, and documented septicemia. Beneficial response to granulocyte transfusions was shown. All patients receiving four to 14 granulocyte transfusions over a period of nine to 14 days recovered from their documented infection. The control group of 19 patients who survived long enough to have received the same number of high blood transfusions, suffered a mortality rate of 70%. In corollary studies granulocytes obtained by continuous flow centrifugation were shown to be qualitatively and quantitatively superior to granulocytes obtained by filtration leukapheresis in terms of phagocytic index, bactericidal capacity, and intravascular survival. Granulocytes collected by either method have been shown to circulate and migrate into areas of inflammation when the recipient does not have preformed antileukocyte antibodies. Survival, migration, and therapeutic effectiveness of granulocytes is markedly reduced in patients with preformed

leukoagglutinins or lymphocytotoxins.

3. Platelet Transfusions

The value of HL-A typing or platelet transfusion support has been clearly documented. HL-A matched siblings show minimal endogenous platelet production. Alloimmunization does not occur in this situation. Phenotypically matched random donors can on occasion serve as a source od non-immunogenetic platelet donors.

Prospective matching of donor recipient not only improves the efficiency of platelet transfusions, but also provides a method for generating monospecific antisera in one-antigen-mismatched donor-recipient transfusion circumstances.

Future Program Plans

The Clinical Oncology Area has developed a strong clinical program oriented towards doing clinical research in specific diseases. The results have indicated important new leads in therapy and the natural history of diseases, particularly in the hematologic malignancies and supportive care. A new challenge now arises to developing approaches in the solid tumors that require the utilization and input from several therapeutic disciplines. Moreover, the medical oncologist must become involved in the diagnosis and management with surgeons and radiotherapists in the early patient rather than restrict his efforts to the end stage patient. Important new problems arise in getting patients and referrals, in studying the interaction of modalities and shortening the rather long-term follow-up needed in these more slowly growing malignancies. We must also consider other approaches to the control of neoplastic cell growth such as the use of natural cell regulators (i.e., growth conditioning factors of human leukocytes) immunotherapy, and use of viral inhibitors to prevent reinduction. In the near future, consideration must be given to the design of more rational therapies to use specific resistant drugs that add toxicity. A more laboratory-oriented program tied into the clinical studies must be considered as important to future progress Some of these programs include the measurement of binding proteins, and hormone assay methods to measure drug blood and tissue levels, and culture or biochemical techniques to study specific sensitivities of tumor cells to anti-neoplastic drugs. We, therefore, need additional laboratory space and people willing to work with clinical material.

The supportive care-protected environment-transfusion program has always taken a great deal of our resources and is now expanding into several of the Chemotherapy Program units as well as a large contract program in tissue typing, and sterile supplies. We need to apply these techniques in a coordinated way and include other areas, particularly the solid tumors. Additional clinical contracts are envisioned closely tied into our intramural programs to facilitate more rapid solutions to clinical problems of support therapy.

A bright spot in the past year has been the activities of Dr. R. Simon to provide automated help to our senior staff in data collection and analysis. We now have an effective data collection system that is being expanded to all our program areas providing them with a more efficient way of collecting and analyzing data. In the future, this area can readily be made into a functioning unit in our program.

Finally, the clinical program area has provided trained oncologists to other centers. Our expanded clinical efforts properly utilized provide a valuable national resource for trained clinical oncologists. The American Board of Internal Medicine will provide a certification examination in medical oncology. A more formal training program will be offered to our Clinical Associates and fellows beginning in July 1972 - involving all of our clinical resources. This will also provide a trial program in the event that the draft will expire and we must compete with universities and other training programs.

Honors and Awards:

Paul P. Carbone, M.D.

1. Visiting Professor at Stanford University Medical Center and Veterans Administration Hospitals, Stanford, California, during the month of December 1971.

2. "Breast Cancer: A Challenging Problem", presented at the University of Chicago Hospitals and Clinics, March 1972.

3. Invited to serve on Oncology Test Committee of the Board of Internal Medicine, 1972.

4. Medical Consultant with the Intramural Committee of the Department of Medicine, Massachusetts General Hospital, April 1972.

5. "Lymphomas other than Hodgkin's Disease", presented at the American College of Physicians' postgraduate course held in Buffalo, New York, June 1972.

6. Member, Advisory Committee on Epidemiology, Diagnosis and Therapy of Cancer of the American Cancer Society, 1971-74.

7. President-elect, American Society of Clinical Oncology, 1972-73.

8. Consultant to the American Joint Committee on Staging End Results for the year 1971-72.

9. Chairman, Hodgkin's Disease Task Force, American Joint Committee.

10. Participated in the International Symposium on Hodgkin's Disease held at Stanford University School of Medicine, March 20-24, 1972.

11. "Combination Chemotherapy for Lymphomas (Excluding Hodgkin's Disease)" presented at the National Conference on Cancer Chemotherapy, New York, June 1972.

Publications:

1. Carbone, P. P., Ziegler, J. L., Morrow, R., Kyalwazi, S., Brown, C. H., DeVita, V. T., and Berard, C.: Burkitt's tumor: A comparative study in Africa and the United States. In, Recent Results in Cancer Research Current Concepts in the Management of Leukemia and Lymphoma, vol. 36, Ultmann, J. E., Griem, M. L., Kirsten, W. H., and Wissler, R. W. (Eds.) New York, Springer-Verlag, 1971, pp. 126-136.

2. Levine, P. H., Ablashi, D. V., Berard, C. W., Carbone, P. P., Waggoner, D. E., and Malan, L.: Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. Cancer 27: 416-421, 1971.

3. Kyle, R. A., Heath, C. W., and Carbone, P. P.: Multiple myeloma in spouses. Arch. Intern. Med. 127: 944-946, 1971.

4. Carter, S. K., and Carbone, P. P.: Current studies in hematologic malignancies. In, Cancer Chemotherapy, Elkerbout, F., Thomas, P., and Swaveling, A. (Eds.). Leiden University Press, 1971, pp. 64-83.

5. Carbone, P. P., and DeVita, V. T.: Combined chemotherapy of Hodgkin's disease. In, Cancer Chemotherapy, Elkerbout, F., Thomas, P., and Zwaveling, A. (Eds.). Leiden University Press, 1971, pp. 271-278.

6. Carbone, P. P., Kaplan, H. S., Musshoff, K., Smithers, D. W., and Tubiana, M.: Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res. 31: 1860-1861, 1971.

Serial No. NCI-3422
1. Office of the Associate Scientific Director for Clinical Oncology
2. Uganda Cancer Institute
3. Makerere University Kampala, Uganda

PHS-NIH
Individual Progress Report
January 1, 1971 through December 31, 1971

Project Title: Study of Burkitt's lymphoma, Hodgkin's disease, malignant melanoma, Kaposi's sarcoma, and hepatocellular carcinoma

Previous Serial Number: Same

Principal Investigators: John L. Ziegler, Charles L. Vogel, Aron Primack, Avrum Z. Bluming, David S. Sherer

Other Investigators: Sebastian K. Kyalwazi, Ian W.C. McAdam, Charles L.M. Olweny, and Ian T. Magrath

Cooperating Units: Pathologic Anatomy Branch, NCI; Immunology Branch, NCI; Laboratory of Chemical Pharmacology, NCI; Departments of Surgery, Pathology and Medicine, Makerere University, Kampala, Uganda

Man Years:

Total:	10
Professional:	5
Other:	5

Project Description:

Objectives:

The Uganda Cancer Institute, established in 1967, has a fourfold purpose: (1) to provide clinical and investigative research facilities for collaborative cancer research between Makerere University Medical School, the National Cancer Institute, and other cancer research centers throughout the world; (2) to establish a postgraduate training program in clinical and investigative oncology; (3) to provide an active oncology consultative service for local and upcountry hospitals; and (4) to carry out multi-disciplined studies of common cancers in Uganda in a search for etiology,

pathogenesis, and effective treatment. To these ends the Lymphoma Treatment Center was created in 1967 from the former Ward 4 at Old Mulago Hospital, generously made available by the Ministry of Health. This center was soon joined by a sister unit, the Solid Tumor Center, creating a total of 34 cancer research beds for both pediatric and adult neoplasia. Together, the wards and associated laboratories form the Uganda Cancer Institute. Staffed by advisors from the National Cancer Institute, these wards admit cancer patients from Mulago and upcountry hospitals for investigation and treatment according to specific research protocols. These studies, prepared jointly by collaborators from the Makerere University and Government Medical Staff and the National Cancer Institute team, are first reviewed by the Uganda Cancer Institute Committee, and then sent for final approval by the National Research Council and the National Cancer Institute. Details of specific investigations completed or underway will be given below. In addition to the numerous local and regional collaborative studies, joint projects have been established between the Uganda Cancer Institute and the NIH, as well as a number of international research centers.

Efforts to establish an oncology training program have met wtth considerable success, due largely to the cooperation and support from the Medical School, and to the generous donation of a Research Fellowship from Kilembe Mines, Kasese. The Fellowship is intended to provide intensive training in the clinical and investigative oncology for postgraduate Ugandan physicians and surgeons. This year there are several qualified Ugandans applying for the post.

Dr. C. Olweny, a lecturer in the Department of Medicine, will soon be completing a two-year postgraduate training period in oncology at the National Cancer Institute in Bethesda, and will thereafter join the full-time professional staff at the Uganda Cancer Institute. A Ugandan Ph.D. candidate at the University of Indiana, Dr. Andrew Okot, has also applied to join the research staff of the Institute, where he hopes to continue his studies of viral oncogenesis. Other postgraduate students in medicine and surgery have indicated an interest to undergo further training in oncology. The interest and enthusiasm for pursuing a career in cancer research by local physicians has greatly encouraged and stimulated the training program, and support for another fellowship is currently being negotiated.

On the undergraduate level, the clinical clerkship available during the medical school holidays has become highly competitive with as many as thirty student applications for four clinical posts. In addition, clerkships are sought by medical students from the U.S.A. and Britain during periods when Makerere Medical School is in session.

Finally, regularly scheduled rounds, conferences, tutorials and seminars in clinical and investigative oncology are held in the Medical School throughout the year, and participation in upcountry and regional meetings, particularly the quarterly conferences held by the Uganda Medical Association, and the annual meetings of the Associations of Physicians and Surgeons of East Africa, are attended and supported by the Institute staff. All of these educational efforts assist in disseminating the results of research projects, and in involving the Medical Community, both locally and regionally, in the overall research effort.

On July 5-7, 1971, a Symposium on Hepatocellular Carcinoma was held in Kampala under the joint auspices of the Uganda Cancer Institute, the National Cancer Institute, and the International Agency for Research on Cancer. Following the conference, a Planning Session was held including members of the Uganda Cancer Institute, representatives from the Ministry of Health, the National Research Council, and a number of visitors representing many cancer research institutions. This Committee was assigned to review the history, present status, and future plans of the Uganda Cancer Institute.

I. Lymphoma Treatment Center

The Lymphoma Treatment Center has become a nation-wide referral center for pediatric patients with neoplasia. In the past year 95 new patients were admitted to the Lymphoma Treatment Center for the following diagnoses:

Burkitt's lymphoma	34
Embryonal sarcoma	4
Fibrous dysplasia	2
Hodgkin's disease	16
Tropical splenomegaly syndrome	9
Lymphoblastic lymphoma	4
Kaposi's sarcoma	1
Acute lymphoblastic leukemia	2
Sickle cell disease	1
Reticulum cell sarcoma	1
Retinoblastoma	1
Giant follicular lymphoma	1
Histiocytic lymphoma	2
Acute myeloblastic leukemia	1
Abdominal sarcoma	1
Neuroblastoma	1
Miscellaneous diseases	14
Total	95

The majority of these patients were fully evaluated and treated according to the study protocols to be described, the major research emphasis being on the management and treatment of Burkitt's lymphoma.

A. Burkitt's Lymphoma

1. Treatment

The results of a randomized clinical trial which compared single with multiple doses of cyclophosphamide (CTX) showed that patients with localized tumors did well on either regimen, but that patients with generalized disease required multiple doses to establish a durable remission. Relapse in this latter group, however, tended to be drug-resistant, though responsive to other agents such as vincristine (VCR), methotrexate (MTX), and cytosine arabinoside (ARA-C). A new trial was initiated in September, 1970 which compared six doses of CTX with a sequential regimen (TRIKE) consisting of CTX, VCR/MTX, ARA-C, CTX, VCR/MTX, ARA-C given at two-week intervals at random to patients with generalized tumors. The results are summarized in the following table.

Treatment Group	No. Treated	No. Relapse	(%)
Multiple-Dose CTX			
Stage III	17	12	(71%)
Stage IV	7	5	(71%)
Total	24	17	(71%)
TRIKE			
Stage III	12	9	(75%)
Stage IV	6	2	
Total	18	11	(61%)

Thus the institution of different drugs in the early treatment of Burkitt's lymphoma did not result in a significant reduction of relapse frequency. Remission duration, moreover, was more prolonged in the CTX-treated group, although survival is similar in both groups. The full data has been submitted for publication (see references).

In February 1971, a new protocol for the treatment of Burkitt's lymphoma was devised. This study was the result of a comprehensive review of the nature of relapse in Burkitt's lymphoma. Briefly, relapse was found to consist of two distinct types, early and late. Early relapse was characterized by recurrent tumor growth, usually within the first 10 weeks following initial therapy, associated with a high frequency of central nervous system (CNS) involvement, and only partially sensitive to continued drug therapy. These patients had a poor prognosis and nearly all patients have died. In contrast, late relapse occurred well beyond the initial 10-week interval, and was characterized by tumor appearance in previously uninvolved anatomic sites, sparing of the CNS, and a good prognosis. It was apparent in this analysis that intensive or minimal chemotherapy appeared to have no effect on the nature or frequency of relapse. It was also noted that relapsing patients tended to be anergic to recall antigens and to autologous tumor extracts. It was thus decided that immunotherapy should be given a trial in Burkitt's lymphoma. The most reasonable immunotherapeutic approach was the use of BCG administered by the method of Mathe which was shown in other studies at this Institute to significantly potentiate delayed hypersensitivity responses.

Accordingly, a new protocol was designed to achieve clinical remission using two doses of intravenous cyclophosphamide 40 mg/kg, following which patients in remission were randomized by stage to receive BCG immunotherapy or no further treatment. Immunotherapy was administered by the scarification technique every 4 days for 4 weeks and then weekly for 6 further weeks. Patients on immunotherapy and controls underwent periodic skin testing before, during and after immunotherapy using recall antigens, and autologous and allogeneic tumor antigens with appropriate controls. In addition, blood samples were collected serially to assess the the effect of BCG on immunoglobulin levels and EB virus titers. The preliminary clinical results of this study are summarized in the following table:

	No.	Relapse (CNS)	Remission Duration (weeks)
BCG	6	4 (2)	7,10,14+,29,31+,32
No BCG	9	6 (2)	5,9,10+,13,14+,14,20+,33,36

It seems apparent from the above preliminary data that BCG affords little protective effect against relapse as compared to no treatment following remission induction. Two patients who relapsed while receiving BCG developed malignant cells in the CSF as the only manifestation of recurrent tumor; this type of relaps may not represent treatment failure, since the CNS is considered an immunological sanctuary.

Analysis of the skin test data is yet incomplete. Enhancement of cutaneous sensitivity reactions to recall antigens was observed in the BCG treated group. There appears to be no correlation between cutaneous sensitivity reactions and the occurrence of relapse.

The problems of neurologic involvement in Burkitt's lymphoma remains a major therapeutic challenge. A large proportion of these patients develop neurologic complications (malignant pleocytosis and cranial neuropathy) as the only manifestation of tumor relapse, and prophylactic therapy has been attempted. An early trial of prophylactic intrathecal chemotherapy showed no protective effect. A second trial currently in progress employs the randomized use of CCNU given with the second dose of cyclophosphamide to patients presenting without neurologic manifestations. Thus far 9 patients have received CCNU and 7 patients hav not. There have been two neurological relapses in each group. Thus it appears that CCNU used in this dosage and schedule does not effectively protect against later CNS involvement, but more patients must be evaluated before firm conclusions can be drawn. At the conclusion of the CCNU trial, a collaborative study has been arranged with the Radiotherapy Center at Kenyatta National Hospital, Nairobi, where patients presenting with neurological involvement will be randomzied to receive prophylactic craniospinal irradiation or no treatment. This study was stimulated by the successful use of this approach in patients with acute lympho cytic leukemia by the St. Jude's group in Tennessee.

Currently the use of systemic hydroxyurea as an adjuvant to intra thecal chemotherapy with cytosine arabinoside (day 1-3) and methotrexate (day 4) is being evaluated in a controlled trial. At the present time eight patients have been randomized to receiv hydroxyurea with intrathecal chemotherapy and eight patients have received intrathecal chemotherapy alone. All patients in both groups have achieved remission and the median follow-up time is 5 months, but the results of this trial are not yet fully evaluated.

Actuarial analysis of survival in 122 evaluable patients followed for the past 4½ years is shown in the table. (Excluded are patients who were admitted in a moribund state, and died within the first week of treatment).

Stage	Tumor Extent	No. Patients	Per Cent Surviving 1 yr	2 yrs	3 yrs
I-II	Localized	31	86%	75%	75%
III	Visceral	66	68%	60%	60%
IV	CNS or Marrow	25	67%	62%	31%

The influence of stage at the time of admission on survival is still evident, and the overall survival is 67%. Relapse in patients in sustained remission for longer than 18 months has not been observed, and these patients may well be considered "cured".

2. Clinical Immunology

Skin tests using recall antigens and autologous and allogeneic tumor extracts have been done in a total of 46 patients at various times during their clinical course. Recent results have confirmed preliminary findings of delayed cutaneous reactivity to autologous tumor antigen extracts, and the correlation of reactivity with disease activity and remission duration. Further results of skin testing suggest that allogeneic cross-reactivity does not occur, even when histocompatibility differences exist. A number of in vitro studies, described below, attempt to confirm and correlate the clinically observed cell-mediated immune reactions in Burkitt's lymphoma.

3. Studies on Cell-mediated Immunity

A micro-technique for lymphocyte transformation using small quantities (0.03 to 0.1 mls per culture) of blood has been chosen as the simplest and most economic method. It does not appear to be any less sensitive than more usual methods of lymphocyte culture, and when DNA replication is assessed in terms of incorporation of tritium labelled thymidine, absolute counts (D.P.M.) in transformed cultures are frequently of the same order as technique using 3×10^6 lymphocytes per culture.

A sensitive in vitro measure of cellular immunity to tumors is the Cr^{51}, cytotoxicity test. In this test tumor cells are labeled with Cr^{51}, incubated in CO_2 with patients' purified lymphocytes, and the amount of Cr^{51} released into the media can be used to calculate the percentage of tumor cells subject to immune lysis. We have tested lymphocytes (peripheral and splenic) from Burkitt lymphoma patients against autologous, allogeneic and tissue culture tumor cells. Although enough data for a conclusive report have not yet accumulated, preliminary observations show a correlation between cytotoxic activity and cutaneous hypersensitivity to tumor cell extracts. Patients will be followed and tested serially during various stages of disease in order to generate more information on the usefulness of the in vitro cytotoxicity test in predicting prognosis. By the use of vital freezing in liquid nitrogen, we have stored batches of cell suspensions from 15 Burkitt lymphoma biopsies. These cells can be used as target cells for further cytotoxicity studies during the patient's subsequent clinical course.

4. Studies on Humoral Immunity

Burkitt lymphoma cells coated with gamma G globulin have been observed by Professor Klein and his colleagues, particularly in patients in relapse, and may represent a form of "blocking antibody." In an effort to ascertain the incidence and relevance of immunoglobulin coating to disease activity and prognosis, an immunofluorescent study to detect the presence of gamma globulins on Burkitt lymphoma tumor biopsies was initiated. Fifteen consecutive biopsies from suspected Burkitt lymphoma patients at the Lymphoma Treatment Center were investigated. The first 10 of these had been previously excised, minced, washed twice in RPMI 1640 media, suspended in 10% DMSO + media and been frozen in liquid nitrogen. The last 5 were freshly obtained, biopsied, processed as above without DMSO or freezing. Totally represented were 11 new tumors and 3 recurrent tumors (2 biopsied were second and third recurrences from the same patient). Eight of the 15 tumors had evidence of speckled immunofluorescence with IgM, probably produced by the tumor cells. Two of the 3 recurrent tumors tested showed strong "ring-like" (coating) immunofluorescence with IgG. No IgA was seen in any of the tumor biopsies. Patients will be followed and recurrent tumors re-tested for immunoglobulin coating in an attempt to ascertain the role of such coating in the disease course and prognosis.

5. Studies on Etiology

Practically all patients with Burkitt's lymphoma have high titers to EB virus (viral capsid antibodies) which persist for a long period of time and bear no apparent relationship to the course of the disease. Viral particles have not been identified in electron microscopic studies of fresh tumor biopsies and the localization of virus in patients in vivo and its mode of spread is unknown. We have studied autopsy tissues from 2 patients with Burkitt's lymphoma using an indirect immunofluorescent technique in order to find evidence of viral antigen. Several high titered anti-EBV (VCA) serums (1:1000) were first diluted to 1:100 and then incubated with cryostat sections of tissues. After washing, FITC goat anti-human gamma globulin was applied to the sections to determine the presence of antibody. No specific fluorescence was noted in any tissue.

6. Cell Kinetics

An in vitro and in vivo study of tumor cell doubling time in Burkitt's lymphoma was recently completed. The potential doubling time was 25 hours, and the growth fraction approaches 100%. The rate of cell loss is estimated to be 70% of the proliferative rate. Analysis of biopsies from 10 patients pretreated with colcemid is underway to estimate the actual doubling time in vivo.

7. Miscellaneous Investigations

A recent analysis of malaria in Burkitt's lymphoma has been completed. The results reveal that patients with Burkitt's lymphoma have findings compatible with a normal immune response to malaria, and as a group reflect the clinical and immunological features expected of a population living in an area of hyper- or holoendemic malaria. The finding of low IgM levels in Burkitt's lymphoma was again confirmed.

Collaborative studies with the East African Virus Research Institute have been initiated to investigate the relation between measles and Burkitt's lymphoma. This study was stimulated by the observation of spontaneous remission in a patient following measles infection. The tumors were found to support the growth poliovirus type 1 or wild measles virus, but no echovirus type 7. Radiobiological studies showed that the polio and measles virus infection resulted in functional destruction of cells. Virus multiplication was observed only in organ cultures, but not in suspended cell culture.

Collaborative studies have been in progress to identify the HL-A phenotypes in patients with Burkitt's lymphoma and controls. This type of genetic analysis may identify a susceptible population of individuals, and may relate to subtle immunological defects. Serum and cells are sent to Dr. Julia Bodmer's laboratory in Oxford for analysis. Results are not yet available.

A study of glucose-6-phosphate dehydrogenase phenotype of Burkitt lymphoma cells is also underway in collaboration with Dr. Philip Fialkow in Seattle. This investigation should provide further evidence for the clonal origin of tumor cells, and be useful in characterizing tumor relapse either as regrowth of the original tumor or new growth of a "reinduced" tumor.

Investigations of chromosomes continue in patients with Burkitt's lymphoma, in collaboration with Dr. Bluming (Columbia University) and Dr. Ulla Gripenberg of Nairobi. It is hoped that the pooled data from the Kampala and Nairobi patients will soon be available for analysis.

Epidemiologic studies in the role of malaria in Burkitt's lymphoma are still in progress, and will be extended as the EBV sero-epidemiologic project in West Nile gets underway this year. The protective effect of hemoglobin AS phenotype has not yet been established. An ancillary project which will soon be completed is an analysis of the effect of hemoglobin phenotype on immunoglobulin levels in children. Finally, a further epidemiologic report of time-space clustering in Burkitt's lymphoma in the Bwamba district was recently published.

B. Other Lymphomas

1. Hodgkin's Disease

Evaluation and treatment of patients with childhood Hodgkin's disease continues and the series is now enlarged to a total of 24 evaluable patients. Recent analysis reveals that there have been 23 complete remissions with MOPP therapy. The patients are divided as follows: stage I-II, 6 patients; stage III, 12 patients,; stage IV, 6 patients. Seven patients, all with stage III or IV tumors, have relapsed after a median remission duration of 15 months (range 9-38 months), and all have responded completely to subsequent MOPP therapy. Two patients in this series have died. Both were in complete remission at the time of death. One died of Herpes simplex encephalitis, the other of severe hookworm anemia and congestive heart failure.

Patients with non-Burkitt's lymphoma have also been evaluated and treated and a recent review of this series has emphasized the distinctive clinical and histological differences from patients with Burkitt's lymphoma. It is now proposed to treat patients with lymphoblastic lymphosarcoma (and other childhood sarcomas) with a combination of cyclophosphamide, vincristine, cytosine arabinoside and prednisone (COAP) in a phase II study. Patients with histiocytic lymphoma will be treated with MOPP combination chemotherapy.

C. Miscellaneous Childhood Tumors

A series of 10 children with embryonal rhabdomyosarcoma have been treated with cyclophosphamide and more recently with cyclophosphamide plus vincristine alternating with actinomycin D. There have been 7 complete and 3 partial responses in this series and the results are currently under review for publication. We have continued follow-up of our 5 surviving childhood patients with Kaposi's sarcoma, 3 of whom remain free of disease and 2 of whom remain in sustained partial remission.

D. Tropical Splenomegaly Syndrome (TSS)

Interest and investigation in the clinical features and pathogenesis of TSS continue. A large kindred of Rwandan immigrants with TSS has been followed for 4 years and a full clinical and immunological evaluation of this family has been submitted for publication. Laboratory studies in TSS have now turned to the role of malaria and immune complexes in the pathogenesis of the disease. Cryoglobulins have been isolated from the patients' sera but not from control sera and on Ouchterlony analysis the cryoprotein contains IgM, IgG and complement. Cryoprecipitation has been found to be complement-dependent and its IgM component contains rheumatoid factor-like activity. Sera from patients with TSS has been found to be hypo-complementemic and anti-complementary. Immunofluorescent analysis of liver biopsies from these patients have identified phagocytosis of gamma globulin, particularly IgM, within the Kupffer cells of the hepatic sinusoids. These cells also contain to a lesser degree IgG and complement. Control liver tissue does not show these findings. Thus far we have been unable to identify malarial antigen or anti-body in the immune complexes or the liver of these patients. The current hypothesis regarding the pathogenesis of TSS is based upon identification of circulating immune complexes which result in particulate stress to the reticulo-endothelial system. The auto-antibody nature of these complexes have been identified. The role of malaria in the pathogenesis of TSS is unclear at the present time and

the reason for amelioration of the syndrome with prolonged malarial prophylaxis is likewise obscure. It is possible that malaria infection stimulates auto-antibody production (rheumatoid factor) which is of sufficient magnitude in certain genetically susceptible individuals (IgM-over producers?) to result in in vivo anti-antibody complexes. Evidence in favor of this is the strong correlation between the presence of rheumatoid factor-like activity, IgM levels, cryoglobulin concentration and the degree of splenomegaly.

Further studies in TSS include the clarification of factors leading to cryoprecipitation, HL-A analysis of patients' lymphocytes and carefully matched neighbor controls, further immunofluorescent investigations, serial studies of patients undergoing chloroquine prophylaxis in order to demonstrate improvement in the various abnormal immunological findings, and a study of the IgG and IgM antibody response to primary and secondary tetanus toxoid immunization in patients with TSS and carefully matched controls.

II. The Solid Tumor Center

In the two years since its opening in December, 1969, 318 patients were admitted to the Solid Tumor Center. Diagnoses of these patients were:

Kaposi's sarcoma	105
Hepatocellular carcinoma	98
Malignant melanoma	54
Hydatidiform mole	21
Choriocarcinoma	8
Miscellaneous diseases	32
Total	318

In general, patients with miscellaneous diagnoses were admitted on the assumption that they had hepatocellular carcinoma. Prominent among these differential diagnostic problems were: metastatic liver disease, (5); liver abscess, (4); bile duct carcinoma, (2); and tuberculosis, (2).

A. Hepatocellular Carcinoma

1. Treatment

At the present time no uniformly effective treatment exists for hepatocellular carcinoma. Most patients die within three to four months of diagnosis and survival of greater than one year in the untreated patient is distinctly unusual. Occasionally patients with disease localized to one lobe of the liver and without unde

lying cirrhosis may benefit from partial hepatic resection. However, it is estimated that only 1% or less of all patients with hepatocellular carcinoma may be cured by surgery. Results of clinical trials of chemotherapeutic agents in hepatocellular carcinoma reported in the literature are generally disappointing. Comparison between trial is difficult, however, because small numbers of patients were involved, careful matching of controls was seldom used, and patient selection for entry to a study was often poorly defined. To standardize future therapeutic trials, a functional and anatomic staging system for hepatocellular carcinoma was proposed. The prognostic validity of this staging system is currently being tested in a computer analysis of survival in 100 patients staged prospectively according to the criteria outlined.

Eight patients have undergone partial hepatic resection. There were two operative deaths. One patient died two weeks post-operatively of progressive hepatic failure. Four patients died of recurrent disease 3, 5, 6 and 18 months post-operatively and one is apparently free of disease at 3 months.

Eleven patients have been treated with intrahepatic arterial infusion with dichloromethotrexate (DCM). Two had prolonged remissions of 9 and 22 months, but both died ultimately of progressive tumor. No other objective responses have been seen.

The results of systemic chemotherapy appear in the table.

Treatment	No. Patients	Response
DCM, Systemic	16	No objective anti-tumor response
CCNU	12	No objective anti-tumor effect
BCNU-5FU	10	No objective anti-tumor effect
No Treatment	53	

2. Etiology

Tests for hepatitis associated antigen (HAA) and antibody to HAA (anti-HAA) were done on serum from 90 Ugandan patients with hepatocellular carcinoma and 224 controls. While there was no difference in the frequency of anti-HAA between these two groups, the frequency of persistent HAA infection was significantly greater in the hepatocellular carcinoma patients than in controls.

Alpha feto protein (AFP) positive hepatocellular carcinoma patients were HAA positive significantly more frequently than AFP negative patients. Although the etiologic implications of these findings are still speculative at the present time, the data provide support for the hypothesis that viral hepatitis infection plays a role in the ultimate development of hepatocellular carcinoma in Ugandan patients. We have also documented a significant increase in HA antigenemia in Uganda patients with macronodular cirrhosis. Further studies aimed at investigating the reasons for the increased frequency are in progress or planned. These include an investigation of host-immune responses in patients with hepatocellular carcinoma, and genetic studies to test Blumberg's hypothesis that the "distribution of persistent HAA in families or in a population is compatible with an autosomal recessive mode of inheritance."

3. Tumor markers

We have now established that there may be as many as four potential biochemical markers of the presence and activity of hepatocellular carcinoma. These include feto protein (AFP), proline hydroxylase, abnormal fibrinogens and human chorionic gonadotrophin.

A rivanol precipitation technique has been developed in our laboratory which increases the sensitivity of AFP detection in the double diffusion test, without apparent loss of specificity. In addition, we have established the electroimmunodiffusion technique for AFP quantitation at the Solid Tumor Center and this test is now in routine use. Of 90 hepatocellular carcinoma patients tested by the standard double diffusion immunologic test, 71% were AFP positive. The quantitative values were spread over a wide range from 0.5 to 4,000 μg/ml with a median value of 71 μg/ml. Twenty-six patients before treatment and 15 patients durin chemotherapy were followed with serial serum AFP determinations. There was a remarkable stability in the quantitative level for each patient which showed little fluctuation during the time they were followed, regardless of the initial level. The only consistent change was a slight tendency for the serum AFP level to rise as the disease progressed. A striking difference was noted in four patients receiving partial hepatectomy who showed a rapid decline in serum AFP and became negative post-operatively.

In collaboration with Dr. K. R. McIntire, (Laboratory of Cell Biology, N.I.H.), further studies in AFP will investigate the possibility of calculating tumor cell number (as done in multiple myeloma patients by Salmon and co-workers) in hepatocellular carcinoma. Preliminary attempts to perfect a radioimmunoassay technique capable of measuring nanogram amounts of AFP are underway; this will facilitate in vitro studies of single cell production rates, coupled with measurements of AFP serum concentrations and in vivo studies of AFP catabolic rate should provide a reasonable estimate of tumor cell number in hepatocellular carcinoma patients.

Results of preliminary studies of the enzyme, proline hydroxylase (PPH) have established that the highest serum levels are found in patients with hepatocellular carcinoma. Serum enzyme assays were done in 89 patients with hepatocellular carcinoma and 118 controls. Seventy-one per cent of patients with hepatocellular carcinoma had abnormal PPH levels (i.e. over 600 units) and 56% had markedly elevated levels (over 1000 units). Only 18% of control patients had abnormal levels and only 7% had levels over 1000. Most of the control patients with abnormal PPH levels had hepatitis. In one patient an objective anti-tumor response to intrahepatic arterial chemotherapy, correlated with a dramatic fall in PPH levels. Currently we are following serial PPH levels in treated and untreated patients to see whether the enzyme might prove to be a useful biochemical index of response to treatment. It is anticipated that continuing studies of PPH in normal, cirrhotic and neoplastic liver tissue might clarify the reasons for the elevations of this enzyme in hepatocellular carcinoma.

Dysfibrinogenemia has been reported occasionally as a complication of hepatocellular carcinoma (HC). Detailed studies of blood coagulation and fibrinolytic enzyme systems were performed in 15 Ugandan Africans with advanced cirrhosis, 29 with hepatocellular carcinoma (14 with and 15 without concomitant cirrhosis) and 20 normal controls. Patients with cirrhosis had decreases in plasma fibrinogen, platelet count and factors of the prothrombin complex and shortening of the euglobulin lysis time. In patients with HC and concomitant cirrhosis mean values for plasma fibrinogen, platelet count and euglobulin lysis time were midway between those of patients with cirrhosis and those with HC. The finding of enhanced fibrinolysis in 7 patients in the present series with HC and concomitant cirrhosis has not been reported previously and could have diagnostic implications in cases where the presence of cirrhosis is uncertain and therapeutic significance where clinical bleeding problems occur. Finally, prolonged thrombin

times in several patients in the present series in the absence of hypofibrinogenemia, circulating heparin-like anticoagulants or increases in fibrinogen degradation products, suggest the possibility that dysfibrinogemia may be more common in HC than previously realized. If this is so it may be possible to quantitate the abnormal protein as another biochemical marker of disease activity.

Braunstein and Ross (Endocrinology Branch, N.C.I.) found human chorionic onadotropin (HCG) by radioimmunoassay in the sera of almost 10% of American patients with hepatocellular carcinoma. Preliminary studies done in Ugandan patients have revealed that 14 of 75 patients (18%) with hepatocellular carcinoma also have HCG in their sera. While these results are preliminary, it appears possible that HCG might be a fourth quantitative marker of disease activity in some of these patients.

4. Ultrastructure

Specimens of neoplastic and non-neoplastic liver obtained at laparotomy were prepared in glutaraldehyde and processed by Dr. G. T. O'Conor (N.I.H.) for electron microscopy and ultrastructural analysis. Seventeen surgical biopsy specimens were studied. The ultrastructural features observed in this series were similar to those previously reported by others. A narrow range in the degree of differentiation of tumor cells was noted but in all cases they were easily recognizable as of hepatic origin. No morphological correlation with the results of serum alpha feto protein was found that no cirus particles were seen in the material examined. Despite the absence of significant new findings in this survey, electron microscopic studies in liver cancer should be pursued but more specifically directed towards the investigation of in vitro cultures and identification of virus therein.

5. Cytogenetics

Karyotypes are prepared from all neoplastic liver specimens, obtained at open liver biopsy, by Mr. Ramon Khiroya, and analyzed by Dr. Avrum Bluming, (Columbia University, New York). Specimens thus far studied have revealed polyploidy, secondary constructions and markers.

6. DCM Uptake and Metabolism Studies

Preliminary studies aimed at assaying neoplastic and non-neoplastic liver tissue for the enzyme, aldehyde (DCM) oxidase, were unsuccessful. Levels of the enzyme in human liver are too low to be measured by the methods used. Currently, specimens of neoplastic and non-neoplastic liver are being obtained at laparotomy serially after a single intravenous injection of 75 mg/m^2 DCM, and assayed by Dr. David Johns (N.I.H.) for its content of intact DCM. The amount of intact DCM in normal and neoplastic tissue should help determine whether the drug is metabolically altered by tumors with different grades of differentiation.

7. Tumor Cell Kinetics

Studies of hepatocellular carcinoma kinetics should provide a better rationale for drug scheduling. Tissue from two patients has already been processed using a tritiated thymidine technique in vitro, and will be analyzed by Professor O. H. Iversen, University of Oslo. Studies are also planned to estimate the growth fraction in vivo.

8. Glutamate Dehydrogenase

Potter and others have studied different subtypes of rodent hepatomas in an effort to define biochemical changes representative of a very early stage in carcinogenesis. The ultimate aim is to find a rodent tumor that could be called "minimum deviation." One enzyme that has been studied in various rodent hepatomas is glutamate dehydrogenase, a catabolic enzyme involved in the oxidation of amino acids. The purpose of the present study is to analyze glutamate dehydrogenase levels in normal liver and tumor tissue obtained from Ugandan patients with hepatocellular carcinoma and to correlate these results with tumor type and degree of differentiation, AFP, HAA, serum and tissue proline hydroxylase, other liver function tests, patient age and sex, and duration of disease. Comparison of these results with rodent data should prove interesting. This study is in progress, but results are not yet ready for analysis.

9. Glucose-6-Phosphatase (G-6-P), Glucose-6-Phosphate Dehydrogenase (G-6-P-D), and Glycogen

Assays of G-6-P, and G-6-P-D have been performed on tumor and non-tumorous liver specimens from patients with hepatocellular

carcinoma in Hong Kong in an effort to explain the frequent occurrence of hypoglycemia in these patients. The results of these clinical studies compared to similar analyses in rodent hepatomas showed that: (1) in both rodent and human hepatocellular carcinoma tissue there was a complete absence of glucose release, and a marked increase in glycolysis via the hexose monophosphate shunt associated with a marked increase in lactic acid production; and (2) glycogen synthesis was markedly diminished in the rodent but was intact in man.

Studies of glucose metabolism are underway in our laboratory in order to provide additional biochemical correlations for the glutamate dehydrogenase study. These results will also be compared with those in the Hong Kong study since it is possible that different etiologic agents may operate in these two areas. Finally, such studies should provide biochemical information that might bear on the clinical problem of hypoglycemia encountered in patients with this disease.

B. Kaposi's Sarcoma

1. Clinical Features

A clinico-pathological classification of Kaposi's sarcoma has been devised delineating four major types.

Two patients with Kaposi's sarcoma complicating pregnancy were the subject of separate case reports. Because of limited bed space only patients with easily evaluable, "florid" or "lymphadenopathic" lesions are being admitted to the Solid Tumor Center. Preliminary clinical trials in patients with "florid" disease established that actinomycin D was superior to cyclophosphamide in this form of the disease. In a subsequent clinical trial patients with "florid" disease were randomized between actinomycin D used alone versus a combination of actinomycin D and vincristine. Although 27 patients are evaluable at present there is no clear difference between the two groups in remission induction and duration. Results are summarized below.

Other agents or regimens evaluated in patients with Kaposi's sarcoma refractory to actinomycin D include: BCNU, a four drug combination (MOPP), Dimethyl imidazole carboxamide griazeno (DTIC, NSC, 45388), Duanomycin and radiotherapy. A summary of results of therapy with these agents to date follows:

Treatment	No. Patients	Response
Act D	14	6 complete remission 6 partial remission 2 unevaluable
Act D + VCR	13	8 complete remission 4 partial remission 1 no response
*BCNU	10	1 complete remission
*MOPP	6	1 objective response
*DTIC	1	1 objective response
*Duanomycin	2	2 no response

*Most patients have received these regimens after failure on other regimens

2. Laboratory Investigations

Studies of lymphocyte transformation in patients with Kaposi's sarcoma have been completed. Tissue culture studies are underway in collaboration with Dr. G. Giraldo, (Institute de Recherches sur les Maladies due Sang, Paris, France). Of 12 tumor specimens, 4 are now well established in tissue culture, 3 are questionable and 2 are still being established. Three were unsuccessful. One established line after 2-3 months in tissue culture has shown herpes-like virus particles on electron microscopy. These particles are similar to those found in another line derived from a Congolese patient with Kaposi's sarcoma. Experiments aimed at characterizing these particles and ascertaining whether they are disease-associated are in progress. Immunologic studies in patients with Kaposi's sarcoma have been reported previously.

C. Malignant Melanoma

1. Chemotherapy

Patients with disseminated or inoperable malignant melanoma have been entered into phase II clinical trials with different agents. In the first trial DTIC (NSC 45388) was used and only one objective response was encountered. Subsequently, a BCNU-VCR combination was tested with equally disappointing results. Thus,

of 16 patients only 2 (13%) had transient, objective evidence o tumor regression. Currently, BTIC (NSC 82196) has been evaluat in eight patients, without significant objective responses to date.

2. Immunologic Studies

In a preliminary report, a correlation between positive skin tests to antigens prepared from autologous tumor and tumor stag was suggested. An expanded study in a larger number of patient however, failed to confirm this preliminary observation.

3. Immunotherapy

Preliminary immunotherapy studies established that Pasteur Institute BCG administered by scarification stimulated cell-mediated immune responses. Additionally, it appeared that such treatment might have beneficial effects as an adjunct to surgic therapy in patients whose disease has not spread beyond the regional lymph nodes. More recent follow-up of the patients in this preliminary study reveals that only one of 6 patients trea with BCG scarification has developed recurrent tumor, one year after completion of immunotherapy. All other comparable patien treated with immunotherapy using a Glaxo BCG preparation administered intradermally relapsed within 6 months of treatmen

Currently, all patients with operable tumors confined to an extremity, with or without regional lymph node involvement, are randomized after local excision and regional lymphadenectomy to receive: (1) BCG scarification; (2) adjunctive CCNU chemo-therapy; or (3) no therapy. To date eleven patients have bee entered on study and of these four have been randomized to rece BCG immunotherapy, five to CCNU and two to no adjunctive therap

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Serial No. NCI-3423
1. Clinical Oncology
2. Office of the Associate Scientific Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: In Vitro Marrow Granulocyte Culture

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone, Joan M. Bull, M. J. Duttera

Other Investigators: Eleanor Stashick, Wilma Stucky, J. Whang-Peng

Cooperating Units: Medicine Branch, CO, C, NCI; Hematology and Supportive Care Branch, CO,C,NCI, and Laboratory of Tumor Cell Biology, ET,C,NCI

Man Years:

Total: 5
Professional: 3
Other: 2

Project Description:

Objectives:

1. Serial Studies of In Vitro Colony Formation in Patients with Acute Lymphocytic Leukemia.

In vitro marrow granulocyte colonies (CFU-c) from patients with acute lymphocytic leukemia (ALL) have been cultured using a methylcellulose system. CFU-c was studied in 8 children with ALL from initial diagnosis through remission with the same chemotherapy combination (prednisone, vincristine, 6-mercaptopurine and methotrexate). The number of CFU-c was mean 0.14/10,000 marrow nucleated cells (range 0 to 0.63) for untreated patients; patients in remission, mean 3.44 (0.17 to 6.10); and for normals, mean 2.3 (0.9 to 5.8). Longitudinal studies revealed a pattern of scant colony formation at initial diagnosis and throughout the period of remission induction until the percentage of lymphoblast fell below 15%, at which time there was a sharp upswing in CFU-c. CFU-c formation overshot the normal range in 3 of 7 patients by the 3rd remission chemotherapy course with a subsequent decline into normal range. Two patients relapsed during study and showed a sharp decline in colony numbers, dropping below normal either just before or at the time of relapse.

Thus, in patients with ALL, colony formation became normal as remission was attained and appeared to be inhibited by an increasing number of leukemic cells in a non-linear fashion.

2. Serial Studies of In Vitro Colony Formation in Patients with Acute Myelocytic Leukemia.

The in vitro marrow granulocyte colonies (CFU-c) of 8 patients with acute myelocytic leukemia (AML) have been studied serially from initial diagnosis into remission with combination chemotherapy. The in vitro method used is a modified methylcellulose system of Till and McCulloch that requires a colony stimulating substance (human embryo kidney supernate). Seven patients revealed a pattern of scant colony formation at the time of diagnosis. The mean number of CFU-c was 0.27/10,000 marrow nucleated cells plated (range 0 to 1.8). The mean CFU-c for 24 normals was 2.3 (range 0.9 to 5.8). A single patient was distinctly different and showed exuberant colony formation at initial diagnosis with CFU-c of 23.6. Three patients achieved a CFU-c greater than 4.0 (range 5.2 to 10.5) by the fourth drug course and have been maintained in clinical remission. Four patients never achieved a CFU-c above 4.0. At the time of first bone marrow remission (blast $\leq$ 5%), 3 of the 4 patients did not reach the normal CFU-c range (0.9). Clinical relapse occurred early in 2 of these 3 patients. Thus in 7 of 8 untreated patients with AML in vitro granulocyte colony formation is impaired. Relapse occurred early in 2 of 4 patients who failed to reach or remain within the normal range. The in vitro colony system may provide a sensitive assay to estimate the completeness and duration of response of patients with acute leukemia who are in apparent clinical remission.

3. Identification of Cytogenetically Abnormal Cells In Vitro in Acute Leukemia.

Twenty patients with previously identified direct cytogenetic abnormalities were examined for persistence of the abnormality in both direct marrow preparations and in vitro cultures. Of 7 patients with persistent direct abnormalities, 4 had these same cytogenetic abnormalities identified in cells from culture. In 3 patients with acute myelocytic leukemia (AML) these abnormalities had been previously associated with leukemic cell lines. Cytogenetic markers in the cultured cells indicate that leukemic cells from some patients can be grown in vitro. As the cells in culture mature, this data supports a block in differentiation theory for AML.

4. Selective Humoral Inhibition of In Vitro Human Granulocyte Colonies Caused by HL-A Incompatible Transfusions.

Studies have shown that prior sensitization to histocompatibility antigen can impair bone marrow engraftment. The ABO red blood cell system in

man may represent an additional transplantation barrier.

We have used a modified methylcellulose system developed by Till and McCulloch to study the effect of ABO, and HL-A incompatible sera on in vitro granulocyte growth of human marrow (CFU-c).

Marrow nucleated cells from normal volunteers were plated with sera from patients with aplastic anemia pre- and post-transfusion, as well as allogeneic normal human sera. When the CFU-c with the addition of colony stimulating factor alone is expressed as 100%, the addition of normal allogeneic serum is stimulatory with a median of 176% (range 125 to 203%). Addition of sera from patients with aplastic anemia never transfused is not different from normal sera with a median of 165% (range 120 to 201%). However, serum from patients with aplastic anemia, multiply transfused and with significant titers of lymphocytotoxic antibody, caused a decrease in colony numbers median 70% (range 42 to 145%) (P value < 0.05).

The effect of selective transfusions on the sera of 2 patients with type O red cells was incubated with donor marrow from HL-A identical, ABO incompatible siblings with type A1 red cells. The anti-A titers were 1:128 and 1:80. There was no inhibition of donor marrow CFU-c with test sera (92, 101%) when compared to controls of autologous donor sera (100%).

In contrast when the sera of a patient with O red cells was incubated with the marrow of a sibling with A1 red cells and HL-A leukocyte type that differed by 1 haplotype, significant inhibition of CFU-c (4%) occurred when compared to autologous donor serum (100%). The CFU-c inhibition was observed only after the patient had received multiple platelet and leukocyte transfusions from the donor sibling.

We conclude that serum with high anti-A titers does not appear to inhibit CFU-c from type A bone marrow, but the humoral inhibition of CFU-c is elicited by repeated transfusion of HL-A non-identical blood products. This humoral inhibitor is not demonstrated in sera prior to transfusion with specific sensitizing cells.

A dog model is used to determine the time of occurrence of specific sensitization by DL-A mismatched blood products. Dogs are transfused weekly with blood from mismatched donor. The sera are then screened against the donor dog's marrow by assaying the in vitro colony inhibition.

Future Course of Project:

1. Additional longitudinal studies of patients with AML and ALL will be initiated following sera inhibitory and stimulatory factors against normal human marrow, and quantitation of granulocyte cluster formation in addition to existing quantitation of > 50 cell CFU with and without various colony stimulatory substances.

2. The CFU-c of Hodgkin's disease patients successfully treated with combi nation chemotherapy will be determined at various times after completic of therapy to compare with normal CFU values. This will be of interest in conjunction with the existing protocol to follow CFU-c sequentially each cycle of chemotherapy.

3. Gallium^{67} is incorporated into mature granulocytes. This property of incorporation will be exploited as a means of specifically labeling in vitro marrow granulocyte colonies with Gallium and enumerating the size and the number of colonies. In addition, it will provide data on the time of transformation of the colonies from granulocyte to macrophage cells. The techniques will also delineate those clones of cells that begin colony formation as macrophages; a differentiation difficult to observe with current counting techniques.

Honors and Awards:

Dr. M. J. Duttera

1. Cytogenetic studies of marrow cultures in vitro. Presented at the American Society of Hematology, San Francisco, December 1971.

2. Serial studies of in vitro colony formation in patients with acute lymphocytic leukemia. Presented at the meetings of the American Associa tion for Cancer Research, Boston, May 1972.

Dr. J. M. Bull

1. Serial studies of in vitro colony formation in patients with acute myelocytic leukemia. Presented at the meetings of American Association of Clinical Research, Atlantic City, April 1972.

Publications

1. Northup, J., Bull, J., and Carbone, P. P.: Quantitative Estimates of Granulocyte Colony Formation in vitro. In In Vitro Culture of Hemopoie- tic Cells, Rijswijk, The Netherlands, 1971, pp. 324-333.

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3. Carbone, P. P., Bull, J. M., Duttera, M. J., and Northup, J. D.: Method of In Vitro Culture of Granulocyte Precursor Cells, Human and Monkey. I In Vitro Culture of Hemopoietic Cells, Rijswijk, The Netherlands, 1971, pp. 464-465.

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5. Duttera, M. J., Gallelli, J. F., Kleinman, L. M., Tanagrea, J. A., and Wittgrove, A. C.: Intrathecal methotrexate. Letter to the Editor, The Lancet 1: 540, 1972.

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9. Duttera, M. J., Carolla, R. L., Gallelli, J. F., Gullion, D. S., Keim, D. E., and Henderson, E. S.: Hematuria and crystalluria after high dose 6-mercaptopurine administration. New Eng. J. Med. (in press).

Serial No. NCI-6308
1. Clinical Oncology
2. Office of the Associate Scientific Director
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biostatistical Center

Previous Serial Number: None

Principal Investigator: Richard Simon, Ph.D.

Other Investigator: Lorraine G. Fitzsimmons, B.A.

Cooperating Units: Hematology and Supportive Care Branch, CO, C, NCI; Medicine Branch, CO, C, NCI; NCI-VA Medical Oncology Branch, CO, C, NCI

Man Years:

Total: 2
Professional: 1
Other: 1

Project Description:

The Biostatistical Center was established during FY 1972 in order to utilize statistical methodology and computer technology to maximize knowledge obtained from clinical trials and other research carried out within the Clinical Oncology Area of the Chemotherapy Program. In addition to direct participation in the design and analysis of clinical trials and other experiments, the Biostatistical Center conducts research in statistical methodology in support of the above goals. A list of projects in which the Center is actively involved follows.

1. Participation in the design of clinical trials. Development of disease and protocol specific evaluation forms for the analysis of clinical trials. Abstraction and computerization of data concerning patient pretreatment characteristics, treatments received, response, toxicity, complications, and follow-up information. Development of computer systems for the acquisition and analysis of this data. Preparation of reports for the monitoring of clinical trials. Thorough analysis of clinical trial experiences.

2. Participation in the design and analysis of retrospective clinical studies. These studies have included survival analyses by diagnosis and year of admission, evaluation of the predictability of liver involvement in Hodgkin's disease patients based upon clinical staging

characteristics, evaluation of the incidence of secondary tumors in Hodgkin's disease patients and its relationship to treatment, correlation of clinical and pathological staging characteristics of lymphoma patients with response to treatment.

3. Abstraction, computerization, and analysis of information concerning the procurement and infusion of granulocytes.

4. Development of a computer system for the storage and reporting of HL-A typing information. Development of computer programs to analyze and report lymphocytotoxic antibodies in patients, and to select platelet or granulocyte donors based upon patient antibodies and HL-A typing.

5. Participation in the design and analysis of laboratory experiments.

6. Development of a computer system for the acquisition and analysis of data from the in vitro granulocyte colony formation assay.

7. Systems analyses of the reporting needs in the clinical environment and development of proposals for utilizing computer technology to meet these needs with a minimum of duplication of effort.

8. Interfacing between the investigators of the Clinical Oncology Area and the computer systems and personnel of the Clinical Center and Division of Computer Research and Technology in order to utilize computerized data available and to eliminate duplication of effort. Obtain retrievals from centralized computer files and develop computer programs to utilize the centralized files for our purposes. Make known to clinical investigators data available, and make known to Clinical Center personnel our priorities for the development of future centralized computer files.

9. Development of stratified randomization schemes and performance of randomizations for clinical trials.

10. Performance of population studies concerning the abnormal frequency of HL-A antigens in patients with particular malignancies.

11. Development of computer programs for the analysis of cell kinetics experiments and utilization of these programs for the analysis of human and animal tumor data.

12. Participation in the computerization and analysis of data concerning the morphology of hematopoietic cells of leukemic patients at various times and the correlation of this data with patient response to therapy and survival.

13. Participation in the design of Phase II clinical trials performed on contract.

14. Development of uniform evaluation forms to be used by all contractors in reporting the results of Phase II studies in carcinoma of the breast. Computerization and analysis of this data.

15. Statistical Research:

    a. Strategies for the introduction of new drugs into treatment combinations.

    b. New methods of analyzing the results of comparative clinical trials in order to reach meaningful conclusions earlier and with fewer patients.

    c. New methods for the comparisons of Poisson processes with application to analysis of infectious episodes.

    d. Models for the inhibition of *in vitro* granulocyte colony formation by malignant cells.

    e. Models for predicting rate of tumor growth and cell kill based upon the concentrations of biological markers.

Serial No. NCI-3403
1. Medicine Branch
(Formerly Solid Tumor Service)
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Clinical Trials and Miscellaneous Clinical Investigations

Previous Serial Number: Same

Principle Investigators: Vincent T. DeVita, George P. Canellos, Robert C. Young, Bruce Chabner, Philip Schein

Other Investigators: Ralph Johnson, James Arseneau, Charles Bagley, Joan Bull, Paul Carbone, Harvey Gralnick, Richard Sherins, Robert Sponzo, Michael Walker, Oleg Selawry, Michael Milder, Steven Larsen, First Year Clinical Associates

Cooperating Units: Radiation Branch, NCI
Baltimore Cancer Research Center
VA Oncology Service
Laboratory of Chemical Pharmacology
Child Health and Human Development Institute
Nuclear Medicine Branch, Clinical Center, NIH

Man Years:

Total:	10.75
Professional:	9.25
Other:	1.5

Project Description:

Clinical trials of the Medicine Branch involves two major areas:

1. New antitumor agents developed through the NCI program are evaluated for toxicity and therapeutic benefit in patients with otherwise untreatable metastatic cancer (phase I studies).

2. Disease Oriented Studies

The Medicine Branch has a disease oriented therapeutic program. The following tumors are being intensively studied:

1. Hodgkin's disease
2. Histiocytic lymphomas (reticulum cell sarcoma)
3. Lymphocytic lymphomas (lymphosarcoma)
4. Burkitt's lymphoma
5. Ovarian carcinoma

6. Carcinoma of the breast
7. Malignant melanoma
8. Chronic myelocytic leukemia

In addition, Phase I studies are conducted in patients with a variety of tumors known to be resistant to available therapies, in an attempt to identify new drugs for the treatment of cancer. Disease oriented therapeutic trials attempt to capitalize on knowledge of existing, somewhat effective therapeutic agents and tumor biology. The latter area is studied in relationship to each disease oriented program and the associated studies should be referred to under miscellaneous clinical investigations, below, and projects entitled cell kinetics and biology (#3420), immunologic aspects of cancer (#3404), physiologic disposition and metabolism of drugs (#6500), and chronic myelocytic leukemia (#3405).

A. Phase I Studies

1. Achronycin

A new oral alkaloid from Eli Lilly is currently under trial. It has demonstrated some interesting animal antitumor effects especially in mouse myeloma. It has generally been free of myeloid toxicity from a dose range of 100 mg/m^2 to 675 mg/m^2 P.O. daily continuous. The main problem is nausea and vomiting due to GI intolerance. We anticipate completion in the coming year.

2. NSC 82196, the imidazole carboxamide derivative

The phase I study of this drug has been completed and the results published. The conclusions reached were that the drug is tolerated at a total dose of 5000 mg/m^2 given over five consecutive days. Myelosuppression, sometimes delayed, has been noted. Nausea and vomiting can be severe. Some evidence for antitumor effect was noted in patients with lymphomas and malignant melanomas.

3. Polyinosinic-cytidylic acid (Poly IC)

The final tabulation of the data of this Phase I trial is being completed. The drug was not found to be overly toxic, acutely, but abnormalities of bone marrow and liver function were noted with chronic use. Interferon induction was obvious but not linear over the dose ranges tested. No antitumor effect was noted.

4. The nitrosoureas

The phase I study with methyl cyclohexyl nitrosourea (MeCCNU) initiated at the time of the last Annual Report and conducted in collaboration with the Baltimore Cancer Research Unit has been completed. MeCCNU is one of several nitrosoureas with activity

against a wide range of animal tumors, but is unique in its high degree of effect against Lewis lung carcinoma. Initial clinical trials were performed with MeCCNU in 24 patients with a variety of advanced tumors. The initial dose (40 mg/m^2) was escalated in 6 steps (3 or more pts./step) to 290 mg/m^2. When possible, the therapy was repeated at the same dose at 6 week intervals. The only acute toxicity was non-dose limiting nausea and vomiting occurring at doses > 170 mg/m^2. Delayed bone marrow toxicity, characteristic of the nitrosoureas, occurred, largely manifest as thrombocytopenia at 4-6 weeks after a single oral dose. Previously untreated patients at 290 mg/m^2 have not consistently had bone marrow suppression, but one previously treated patient demonstrated severe pancytopenia at a single dose of 170 mg/m^2. No evidence of cumulative toxicity was seen in the 9 patients who received multiple doses. No drug-attributable liver or renal toxicity was seen. One patient with metastatic melanoma had an excellent partial remission of skin, subcutaneous lymph node, and bone metastases at 130 mg/m^2. Two patients with brain tumors, one primary and one metastatic melanoma, had neurological improvement suggesting a response. Preliminary clinical trials indicate that MeCCNU is well-tolerated and biologically active when given orally and can be given in previously untreated patients at 220 to 290 mg/m^2. Clinical trials will continue in the next year in collaboration with the Baltimore Cancer Research Unit using the parenteral preparation of MeCCNU prepared in emulsified lipid material.

5. Bleomycin

The Medicine Branch has been involved in two trials with bleomycin. One, a phase I study of the effect of twice weekly dosing in a variety of tumors, conducted in collaboration with the Veterans Hospital Oncology Unit, has been completed. No unique toxicity was identified and side effects appeared related to total dose and the rapidity at which the total dose was approached.

Twice weekly doses of less than 15 mg/m^2 were well-tolerated up to the total toxic dose of about 300 mg/m^2. The use of higher doses led to lower cumulative doses because of the earlier appearance of toxicity. Antitumor activity was noted particularly in patients with lymphomas.

The second trial was conducted using a dose of 25 mg/m^2/day for five consecutive days repeated at monthly intervals in patients exclusively with the lymphomas. Although toxicity was not unique or overly severe at equivalent total doses in the 20 patients so treated, the therapeutic effects have not been improved by this more intensive schedule of drug administration.

elevation of serum transaminase, and renal tubular toxicity with aminoaciduria, glycosuria, phosphaturia, and renal tubular acidosis. The first reported case of major bone marrow depression following treatment with streptozotocin was noted.

The serum half-life of streptozotocin was 15 minutes, with essentially no drug detectable by 2 hours after completion of treatment. Ten to 20 percent of each total dose was excreted in the urine with the N-nitroso group intact. Because of the rarity of bone marrow suppression and some suggestion of antilymphoma effect, a phase II clinical trial in patients with lymphomas has recently bben initiated.

8. Gallium nitrate

A protocol has been prepared for the phase I study of Gallium nitrate in conjunction with the potential predictive use of Gallium scanning. Clinical trials begin in May or June of 1972.

B. Disease oriented programs

1. Ovarian carcinoma

Ovarian carcinoma is the fourth most common killer of women among cancers and the incidence is increasing at the rate of 1% per year. The Medicine Branch program on ovarian carcinoma has been launched by compiling our thoughts and ideas in an extensive review of the subject to be published in the Medical Progress Section of the New England Journal of Medicine. Our in-house efforts include: (a) randomized controlled trial initiated this year of the use of intensive intermittent alkylating agent therapy (cyclophosphamide 80 mg/kg IV q 3-4 weeks) compared to standard therapy with phenylalanine mustard in patients with stages III and IV disease (FIGO classification).

The studies with cyclophosphamide have included the use of radiolabelled drug to examine distribution of active metabolites into ascites fluid; (b) initiation of a phase II effort with hexamethylmelamine, a newer agent of interest, in patients resistant to either programs above to test its activity in alkylating agent resistant patients; (c) a new approach to staging and "second look" operations using peritoneoscopy for both; (d) cell kinetic studies in patients with ovarian tumor in the ascites form.

All of these studies have been recently initiated and results are too preliminary to comment on. Suffice it to say that this tumor is quite sensitive to alkylating agents, and from published literature,

(Smith and Rutledge, Amer. J. Obstet. and Gynec.) it appears that a small fraction of patients with advanced disease (5-10%) may be drug curable. Our goals are to extend this cure rate. To do this the Medicine Branch and the National Cancer Institute have embarked on an extensive contractual arrangement with two institutions with a high volume of new patients with advanced ovarian cancer. These contracts will be initiated before the end of the year. Their major goal is to identify antitumor activity in new and older drugs which might be useful in the treatment of ovarian carcinoma. They will accomplish this by randomly allocating patients to standard therapy or therapy with agents of suggestive usefulness and employ a crossover design to test for cross resistance.

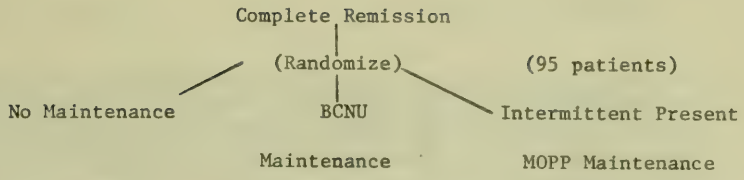
Our goals for future years are to (1) embark on a logically designed drug combination study based on data derived through our contracts. (2) Compare the therapy with a combination to that of the best standard program. (3) Improve methods of evaluating antitumor effect employing peritoneoscopy.

As an additional future goal we plan to reexplore local initiation of antitumor agents into the abdomen, exploiting the principles of volume distribution, absorption, and drug retrieval developed through peritoneal dialysis techniques. The overriding reason for this approach is based on the observation that the great majority of patients with ovarian cancer do not die of distant metastases but of the local abdominal effects of tumor. Studies employing such an approach in the past have been inadequate to draw firm conclusions because of inattention to details of drug dose and volume of distribution.

2. Hodgkin's disease

The drug combination program (MOPP) using vincristine, prednisone, procarbazine and nitrogen mustard, begun in 1963, has continued to provide interesting data. A flow diagram of its progress is shown below:

a. Initial 4 drug pilot study (14 patients) (1963 to 1964)
b. Institution of the current MOPP protocol (1964 to 1967) (43 patients)
c. Institution of randomization to drug maintenance or no maintenance therapy after complete remission. (1967 to present)



d. Other studies parallel to the MOPP program (1967 to present)

1. BCNU therapy in MOPP failures (45 patients)
2. MOPP in previously treated patients (21 patients)
3. MOPP in childhood Hodgkin's disease (18 patients)
4. MOPP versus total lymph node irradiation (TNI) and MOPP plus TNI in stage IIIB patients (36 patients)
5. Other clinical investigations of biology of tumor noted under miscellaneous clinical investigations below.

Our total experience in the drug combination program now encompasses the treatment of 240 patients. The pilot study in 1963 showed that this approach could be used safely and an 80 percent complete remission rate was noted.

In 1964, the current MOPP study was initiated and achieved an 81 percent complete remission rate. Once remission was achieved no further therapy was administered. At the six year point, 71 percent of the complete responders (all stage III and IV patients) are alive. Forty-one percent have remained clinically free of disease since their six months of treatment. These results are contrasted to prior single agent studies with complete response rates of 20 percent, median duration of remission of 12 weeks, median survivals of 2 years and most important 5 year survivals of less than 10 percent most with evidence of active disease.

Since July of 1967 we have randomized complete responders to one of three programs shown in (c) of the flow diagram. The remission rate since then (all stage IIIB patients) continues high (76%). Currently, there is no statistically significant difference between the three arms of the randomization program. This phase of the study should be completed in the coming year.

By all measures, we now have a large number of patients who have remained free of their disease for periods extending as long, in some cases, as eight years.

Our results with BCNU in MOPP failures has confirmed the impression of this drug's activity in the disease. The results have now been published.

A group of patients who relapsed following TNI were treated with MOPP. The results show that they respond in a fashion similar to previously untreated patients and do so with surprisingly little toxicity. Their response duration also appears equivalent to the prior group.

We have collated our experience with childhood Hodgkin's disease and in conjunction with the Radiation Branch are publishing our results. Although significantly better than past reports the results indicate that children under 16 years of age with advanced disease do less well than adults between the ages of 16 and 30 years.

In 1967, because of a lack of agreement on the proper approach to patients with stage IIIB disease, patients with this stage were randomly allocated to either MOPP or TNI. MOPP faired favorably. However, patients treated with TNI (median survival 8 mos.) tended to recur and die with disease outside nodal areas and patients treated with MOPP (median survival > 48 mos.) tended to recur in previously involved lymph nodes. For the past two years a study has been in progress using both modalities in the sequence MOPP-TNI in patients with stage IIIB disease. The results are not yet sufficient for comment. The patients on the MOPP program have had continuous evaluation to assess the long term effects of treatment on the long survivors and will be discussed below and under the section on immunology and cancer (#3404).

Our future goal is to prevent relapses in the high percentage of complete responders. We feel we can best employ our resources to this end by attempting to evolve another effective combination different than MOPP which can be employed in tandem.

3. Lymphocytic and histiocytic lymphomas

The Medicine and Radiation Branches have independently developed treatment programs which appear optimal for the treatment of the non-Hodgkin's lymphomas and may offer two forms of therapy for some stages. To evaluate the efficacy of these programs, we have embarked on a joint randomized trial which randomizes patients, stage by stage to one of the two therapeutic programs. The first results should be available for analysis by next year.

The results with our program employing Vincristine, Cyclophosphamide, and prednisone (CVP) in 5-day courses in a non-randomized group of previously untreated patients have been published this year.

The histiocytic lymphomas (reticulum cell sarcoma) are a particularly virulent type of lymphoma included in the above randomization scheme, but treated with modified MOPP. This approach is based on our observation, now being assembled for publication, that a small percentage of patients (20 percent) who achieved a complete response with this program in the past have not had recurrences up to 6 years post-treatment. The response and survival in this disease is particularly related to the organ site of involvement. Our goals in the coming year are to assemble a new drug combination or improve on the old one by including such new agents as Adriamycin or Streptozotocin.

An extensive evaluation of the correct approach to staging the non-Hodgkin's lymphomas is in progress (See miscellaneous clinical investigations below).

4. Burkitt's lymphoma

The Burkitt lymphoma study continues to provide data on long-term survival of the American variety which, stage by stage, appears comparable to its African cousin. These patients are also studied intensively for information on other relevant studies such as Epstein-Barr virus titers and histocompatability antigenic makeup. (See miscellaneous clinical investigations below.)

5. Carcinoma of the breast

a. Adjuvant chemotherapy

The use of chemotherapy as an adjuvant to surgery for curative intent is currently under investigation. Patients with stage II disease (axilla positive) are entered on a randomized trial between placebo and l-phenylalanine mustard. The treatment is continued until evidence of recurrence of metastatic disease.

b. Advanced metastatic disease

For patients with far-advanced endocrine refractory disease, we have initiated a cyclic quadruple combination chemotherapy program which includes 14 days of oral cytoxan, 100 mg/m^2, and prednisone, 60 mg/m^2, in addition to parenteral 5-FU, 700 mg/m^2, and methotrexate, 70 g/m^2 on days 1 and 8. After a 2-week rest period, off medication, the cycle is restarted for a minimum of six monthly cycles. Fifteen patients have been entered so far with 13 evaluable at present. Seven patients achieved a good response with disappearance of soft tissue disease and/or stabilization of bone lesions. Six who failed are all dead with a

mean survival of 5.6 months. Of the responders, one has died at 10 months, the others are living with a mean survival of 12 months from onset of therapy (three remain in their first remission). The toxicity has been tolerable, especially in the absence of hepatic disease.

6. Chronic meylocytic leukemia

See separate section on this disease.

7. Malignant melanoma

A significant number of patients with metastatic malignant melanoma will continue to be admitted for therapy with newer antitumor agents. Activity, although currently of minor degree, has been demonstrated over the year with the nitrosoureas, including the most recent derivative, MeCCNU, and the imidazole carboxamide, NSC 82196.

C. Clinical investigations, miscellaneous

1. Staging in lymphomas

A controversy has arisen concerning the proper approach to staging of lymphomas. With Hodgkin's disease this has eventuated in the widespread use of staging laparotomy. The indications for such an approach in the non-Hodgkin's lymphomas is less clear. To avert the unnecessary use of staging procedures, a collaborative effort of the Medicine and Radiation Branches is underway to evaluate each staging procedure in sequence to assess the ultimate value of the next procedure in line in yielding positive studies when the prior test is negative.

The results are preliminary, but tend to indicate that, as contrasted to Hodgkin's disease, the non-Hodgkin's lymphomas are often so widespread that simple staging procedures may supplant laparotomy. In the process of evaluating such procedures, we have performed 100 peritoneoscopies on patients with lymphomas and have clearly defined its role in staging. As a procedure for examining and performing multiple biopsies of the liver, it is equal to examination of the liver at surgery, in terms of yield of positive diagnoses. When peritoneoscopy and liver biopsies are negative, subsequent laparotomy rarely indicates liver involvement. Since the procedure requires one day of hospitalization, compared to 10 days for laparotomies performed for the same purpose, its use simplifies staging considerably The results of the procedure in these 100 consecutive patients are being prepared for publication.

2. Reproductive capacity of patients successfully treated with drugs for malignancy

The study initiated last year has been completed. Seventy percent of men successfully treated for lymphomas with MOPP or CVP are coincidently sterilized. In the coming year, we are allocating such patients to testosterone and estrogens prior to chemotherapy to evaluate our ability of inhibit spermatogenesis, hormonally, and prevent drug induced damage. The study has also been extended to the examination of the reproductive capacity of treated women, but the results are too preliminary at present, and will be reported on next year.

3. Long-term drug effects on bone marrow in successfully treated lymphoma patients.

A study has been initiated using the technique of in vitro culture of bone marrow cells to assess the long-term effect of our more successful drug treatment programs on bone marrow functions as measured by marrow colony forming units.

4. Second tumors in long survivors with Hodgkin's disease

In the past year, three second tumors have been observed in young patients with Hodgkin's disease. An examination of second tumors in our entire population of patients with Hodgkin's disease treated with all varieties of treatment was prompted by this observation and revealed the following: (1) the incidence of second tumors is about 3 percent in the entire population and in the subgroups treated with (a) standard chemotherapy, (b) standard radiotherapy, and (c) intensive MOPP chemotherapy, (d) TNI radiation (total 465 patients); (2) all three recent second primaries occurred in a small group of 21 patients treated with both MOPP and TNI therapy, a markedly significant increase, in spite of the small numbers. This observation is of significance, because of the tendency to use both these therapies in tandem in earlier stages of disease at various institutions around the country.

5. Protocol for the study of cachexia in malignancy

The cachectic state in patients with malignancy is poorly understood and occasionally irreversible, even when an antitumor effect has been achieved. Because of recent interest in the use of hyperalimentation in patients unable to consume sufficient calories to maintain body weight, a study has been initiated to examine metabolic derangements in cachectic patients.

Honors and Awards:

Dr. DeVita

1. Appointed Associate Professor of Medicine, George Washington University School of Medicine, July 1971.

2. "Combination Chemotherapy." Presented at Workshop -- Annual Meeting of American Society of Clinical Oncology, March 1972.

3. "Management of Stage III Ovarian Carcinoma." Presented at Workshop -- Annual Meeting, American Society of Clinical Oncology, May 1972.

4. Lectures on "The Use of Drugs in Combination for the Treatment of Cancer," "The Cure of Cancer with Drugs," and "Hodgkin's disease -- Panel Discussion," all presented at Fifth Joint Latin American Cancer Congress, October, 1971.

5. "Lecture - Chemotherapy and Management of Lymphomas," and "Panel Discussion - Treatment of Cancer," presented at Annual Medical Assembly, Ponce, Puerto Rico Medical Society, Nov., 1971.

6. "Hodgkin's disease." Presented at Recent Advances in Hematology and Cancer Chemotherapy, Orange County Medical Society, Dec., 1971.

7. "Chairman, Workshop in the Management of Lymphomas." American Society of Hematology Annual Meeting, Dec., 1971.

8. "Staging and Chemotherapy of Ovarian Carcinoma." Presented at Course -- Current Concepts in the Management of Malignant Disease, May 1972, NIH.

9. "Lecture - The Use of Drug Combinations in the Treatment of Hodgkin's disease." Presented at National Conference on Cancer Chemotherapy, sponsored by NCI and American Cancer Society, June, 1972.

10. "The Chemotherapy of Lymphomas." Presented at the New York Society for the Study of Blood, March 1972.

11. "The Chemotherapy of Lymphomas." Presented at the Fourth Annual Southwestern Wisconsin Cancer Congress, March 1972.

12. "Chemotherapy of the Lymphomas," and "Bone Marrow Transplantations and Platelet Transfusion." Presented at Third Annual Cancer Symposium, Mt. Zion Hospital and Medical Center, Jan., 1972.

13. "Chemotherapy of Lymphomas," and "Chemotherapy of Ovarian Cancer." Presented at Sixth Annual Cancer Symposium, Akron City Hospital, March 1971.

14. "The Role of Chemotherapy in the Management of Patients with Lymphomas." Presented at Pacific Northwest Research Foundation, Seattle, Wash., May, 1971.

15. "Lecture - Chemotherapy of Lymphomas." Presented at Postgraduate Course on Modern Clinical Cancer Chemotherapy, Mt. Sinai Hospital and Medical School, May 1971.

16. "The Management of Patients with Hodgkin's disease." Presented at Medical Grand Rounds, New York Hospital, Cornell Medical Center, Oct., 1971.

17. "Chemotherapy of Lymphomas." Presented at American College of Physicians Postgraduate Course, Memorial Hospital, New York, October 1971.

18. Participated in "Seminar on Lymphomas." Sponsored by American Cancer Society, and St. Vincent's Hospital, Jacksonville, Florida, Nov., 1971.

19. "Lecture - The Treatment of Lymphomas." Presented at Annual Cancer Seminar, State of Maine, Waterville, Maine, June 1971.

20. "Lecture - Chemotherapeutic Care of Hodgkin's disease." Presented at Long Island Jewish Medical Center Symposium on Hodgkin's disease, Oct., 1971.

21. "Chemotherapeutic Implications of Staging in Hodgkin's disease," and "Peritoneoscopy in the Staging of Hodgkin's disease." Presented at Conference on the Staging of Hodgkin's disease, Ann Arbor, Michigan, April 1971.

22. "Combination Chemotherapy of Hodgkin's disease," "Intensive Chemotherapy of Hodgkin's disease; long-term complications." and "Workshop - The Future Role of Chemotherapeutic Trials in Hodgkin's disease." All presented at International Symposium on Hodgkin's disease, Stanford University, March 1972.

23. "Management of Ovarian Cancer with Drugs." Presented at American Cancer Society Symposium on Gynecologic Cancer, Anne Arundel Hospital Annapolis, Md., Feb., 1972.

Dr. Canellos

1. "Treatment of Lymphomas." Presented at Raleigh Academy of Medicine, Oct., 1971.

2. "Chemotherapy of Solid Tumors." Presented at Doctor's Hospital, June 1971.

3. "Combination Chemotherapy for Cancer." Presented at Harvard Medical School, Dec., 1971.

4. "Treatment of Leukemia, Lymphoma." Presented at Clinical Pharmacology Seminar, University of Virginia, Dec., 1971.

5. "Combination Chemotherapy for Breast Cancer." Presented at Peter McCallum Clinic, Melbourne, Australia, March 1972.

6. "Treatment of Lymphoma." Presented at McMaster University, Hamilton, Ontario, June 1971.

7. "Treatment of Lymphoma." Presented at University of Alberta, Canada, June 1971.

8. "Treatment of Myeloma." Presented at Doctor's Hospital, Feb., 1972.

9. "Survival Following Chemotherapy for Hodgkin's disease." Presented at Hodgkin's disease Meeting, Oct., 1971.

10. "Treatment of Lymphoma." Presented at Philadelphia General Hospital, May 1972.

11. Visiting Professor of Medicine, University of Colorado Medical School, June 1972.

12. "Metabolic Complications of Malignancy." Presented at George Washington University, Department of Pharmacology, May 1972.

13. "Treatment of Testicular Cancer." Presented at Walter Reed Hospital, June 1972.

14. "The Hematologic Malignancies, I, II." Presented at Georgetown University School of Medicine, Feb. 1972.

15. Participated in Seminars in Hematology, Georgetown University, School of Medicine, 1972.

Dr. Milder

1. Milder, M.S., Larson, S.M., Bagley, C.M., DeVita, V.T. and Johnston, G.S.: The liver spleen scan in Hodgkin's disease.Presented at the 19th Annual Meeting of the Society of Nuclear Medicine.

Dr. Larson

1. Larson, S.M., Milder, M.S., Tretner, I.H., Jones, I.A.E., DeVita, V.T., Henderson, E.S., Graff, K.S., and Johnston, G.S.: Ga^{67} photoscanning in lymphoma and leukemia.

Dr. Schein

1. "Lymphomas and Hodgkin's disease." Presented at Symposium on Advances in Cancer Therapy, Philadelphia Department of Health, May 1972.

2. To present four talks on "Current Concepts in Chemotherapy," at the Cancer Symposium, Enoch Calloway Cancer Clinic, La Grange, Georgia, April 1972.

3. "Cancer Chemotherapy: Current Concepts and Results." Presented at an NIH Lecture Series on Current Research in Oncology, Jan., 1972.

Dr. Young

1. Young, R.C., Canellos, G.P., Schein, P.S., Chabner, B., Walker, M., and DeVita, V.T.: Initial clinical trials with methyl-CCNU 1-(2 chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU), presented at the Annual Meeting of the American Association for Cancer Research, Boston, Mass., May 1972.

2. "Treatment of the Carcinoma of the Breast and Ovary." Presented at Doctor's Hospital, March 1972.

3. "Hodgkin's disease." Presented at Letterman General Hospital, San Francisco, California, March 1972.

4. "The Treatment of the Acute Leukemias." Presented at Letterman General Hospital, San Francisco, California, March 1972.

5. "Chemotherapeutic Approaches to the Treatment of Hodgkin's Disease." Presented at Teaching Symposium on Clinical Oncology, Sponsored by the Medical College of Virginia, Williamsburg, Va., March 1972.

6. "Effects of Chemical Therapy on Hodgkin's disease." Presented at the University of Pennsylvania, Philadelphia, Pa., April 1972.

7. "Chemotherapy of Hodgkin's disease and the Lymphomas," Wichita, Kansas, May 1972.

8. "Treatment of Hodgkin's disease and Lymphoma." Presented at The Society of Internists, Louisville, Ky., May 1972.

Publications:

1. Carbone, P.P., Ziegler, J.L., Morrow, R., Kyalwazi, S., Brown, C.H., DeVita, V.T., and Berard, C.: Burkitt's tumor: A comparative study in Africa and the United States. In Ultman, J.E. (Ed.) Recent Results in in Cancer Research - Current Concepts in the Management of Leukemia and Lymphoma. New York, Springer-Verlag, 126-136, 1971.

2. DeVita, V.T.: Chemotherapy of Lymphomas. In Ultman, J.E. (Ed.) Current Concepts in the Management of Leukemia and Lymphoma. New York, Springer-Verlag, 159-171, 1971.

3. DeVita, V.T.: BCNU (1,3 bis chloroethyl-1-nitrosourea: Clinical studies and pharmacology. In Carter, S.K. (Ed.) Proceedings of the Chemotherapy Conference on the Chemotherapy of Solid Tumors. An Appraisal of 5-Fluoroura and BCNU. 181-183, 1970.

4. Young, R.C., DeVita, V.T., Serpick, A.A., and Canellos, G.P.: The treatment of advanced Hodgkin's disease with 1,3 Bis (2-chloroethyl)-1-nitrosourea) BCNU. New Eng. J. Med. 285: 475-479, 1971.

5. Carbone, P.P., and DeVita, V.T.: Combined chemotherapy of Hodgkin's disease. In Elkerbout, F. (Ed.) Cancer Chemotherapy. The Netherlands Leiden University Press, 271-279, 1971.

6. Bagley, C.M., DeVita, V.T., Berard, C.W., and Canellos, G.P.: The chemotherapy of advanced lymphosarcoma: Intensive cyclical combination chemotherapy with cyclophosphamide, vincristine and prednisone. Ann. Int. Med. 76: 227-334, 1972.

7. Bagley, C.M., Roth, J.A., Thomas, L.B., and DeVita, V.T.: Clinicopathologi correlations of liver biopsy in 127 patients with Hodgkin's disease. Ann. Int. Med. 76: 219-225, 1972.

8. Van Thiel, D.H., Sherins, R.J., Myers, G.H., and DeVita, V. T.: Evidence for a specific seminiferous tubulur factor affecting FSH secretion in man. J. Clin. Invest. (In press)

9. DeVita, V.T., Arseneau, J.C., Serins, R.J., Canellos, G.P., and Young, R.C.: Intensive chemotherapy for Hodgkin's disease: Long term complications. J. Nat. Cancer Inst. (In press)

10. DeVita, V.T.: Use of procarbazine in combination with other drugs in the treatment of advanced Hodgkin's disease. In Carter, S.K. (Ed.) Proceedings of the Chemotherapy Conference on Procarbazine (Matulane) NSC 77213 Development and Application. National Cancer Institute, 1970.

11. DeVita, V.T.: Rationale for the use of drugs in combination for the treatment of cancer. Proceedings of the V Joint Latin American Cancer Congress, Caracas, Venzuela, Oct., 1971. (In press)

12. Canellos, G.P., Young, R.C., Berard, C.W., and DeVita, V.T.: Effect of combination chemotherapy on the survival of patients with advanced Hodgkin's disease. Arch Int. Med. (In press)

13. Bagley, C.M., Canellos, G.P., Young, R.C., Gallelli, J.F., and DeVita V.T.: Clinical trials with intravenous imidazole 5-carboxamide 5 [3-3 Bis 2-chloroethyl)-1-triazeno] NSC 82196. Cancer Chemother Rep. (In press).

14. Bagley, C.M., Young, R.C., Canellos, G.P., and DeVita, V.T.: The treatment of ovarian carcinoma, possibilities for progress. New Eng. J. Med. (In press)

15. Rosenberg, S.A., Boiron, M., DeVita, V. T., Johnson, R.E ., Lee, B. J., Ultman, J.E., and Viamonte, M.J.: Report on the Committee in Hodgkin's disease staging procedures. Cancer Res. 31: 1862, 1971.

16. DeVita, V. T.: Treatment of Hodgkin's disease in private practice. J. Amer. Med. Ass. (In press)

17. DeVita, V.T., and Canellos, G.P.: The treatment of the lymphomas. Seminars in Hematology. (In press)

18. DeVita, V.T., and Carbone, P.P.: Hodgkin's disease: Current Chemotherapeutic Combinations. Series Hematologica. (In press)

19. Vogel, C.L., Adamson, R.H., DeVita, V. T., Johns, D.G., and Kyalwazi, S.: Preliminary clinical trials of dicliromethotrexate in hepatocellular carcinoma. Cancer Chemother.Rep. (In press)

20. Shimkin, R.M., DeVita, V.T., and Doppman, J.L: Arteriography of ileal carcinoid tumor. J. Canad. Ass. of Radiol. 22: 259-260, 1971.

21. DeVita, V. T.: Combined drug treatment of Hodgkin's disease: Remission induction, duration and survival: an appraisal. J. Nat. Cancer Inst. (In press)

22. Canellos, G.P., Young, R.C., and DeVita, V.T .: Combination chemotherapy for advanced Hodgkin's disease in relapse following radiotherapy. Clin. Pharm. and Ther. (In press)

23. Ohnuma, T., Selawry, O., Holland, J.F., DeVita, V.T., Shedd, D.P., Hansen, H.H., and Muggia, F.: Clinical study with Bleomycin: tolerance to twice weekly dosage. Cancer (In press)

24. Primack, A., Wilson, J., O'Conner, G., Engleman, K., Hull, E., and Canellos, G.P.: Hepatocellular carcinoma with the carcinoid syndrome. Cancer 27: 1182, 1971

25. Bobrow, S., Jaffe, E., and Young, R.C.: Anuria and acute tubular necrosis associated with Gentamycin and Cephalothin. JAMA (In press)

26. Schein, P.S.: Cancer Chemotherapy: Current Concepts and Results, JNCI Monograph. (In press)

Serial No. NCI-3404
1. Medicine Branch
(formerly Solid Tumor Service)
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunologic Aspects of Cancer

Previous Serial Number: Same

Principle Investigators: Vincent T. DeVita, Robert C. Young, Philip S. Schein, Paul H. Levine, John E. Bennett, Arthur S. Levine, Michael P. Corder, Kenneth Graff

Cooperating Units: Viral Leukemia and Lymphoma Branch, NCI; Hematology and Supportive Care Branch, NCI: Clinical Investigations Branch, NIAID.

Man Years:

Medicine Branch:	Total:	1.0
	Professional:	0.85
	Other:	0.15

Project Description:

A. Infections and Cancer

To identify, define, and control infectious complications associated with tumors and the use of immunosuppressive agents in the treatment of patients with cancer.

B. Immunology and Cancer

To study the immunologic status of patients with malignancies and relate these findings to progression or arrest of disease and survival. To exploit the host's immunologic system as an adjuvant to the chemotherapy of tumors. To investigate the presence of a tumor specific antigen in ovarian cancer.

C. Infections

1. Pneumocystis Carinii

Investigations into the identification and treatment of this illness and our published results have been summarized in the Annual Report, 1969-1970. Currently, a new study is underway randomizing patients with known or presumed Pneumocystis infections to Pentamidine therapy

versus Pyrimethamine and Sulfadiazine. This investigation has been prompted by recent reports of the usefulness of Pyrimethamine and Sulfadiazine in this disease, and because a nonnephrotoxic regimen would be of considerable aid in managing these patients.

2. Aspergillus fumigatus

Invasive aspergillosis has been of continuing interest for the past 5 years, and last year studies were completed on the characteristics of the clinical disease caused by various species of Aspergillus. Species identification of the aspergilli responsible for human invasive aspergillosis was accomplished in 39 patients who had cultural and histopathologic identification of Aspergillus at autopsy. Fifty-nine percent of patients had infections caused by A. fumigatus. Thirty-one percent of infections were caused by A. flavus, and 5 percent were due to members of the A. glaucus group.

Forty-two percent of the patients who had A. flavus had pathological lesions of the palate and/or epiglottis, and evidence was presented to suggest that A. flavus may be the leading cause of invasive upper airway aspergillosis.

Two other aspects of the problem of aspergillosis infections are currently being studied. The first is an attempt to assess the significance of fungus cultures in our patient population; the second is an analysis of the X-ray characteristics of invasive aspergillosis.

B. Immunology and Cancer

1. The immunology of Hodgkin's disease

A comprehensive study on delayed hypersensitivity in 103 untreated patients with Hodgkin's disease has been published this year, and the details of the study were reviewed in last year's Annual Report. A second manuscript on PHA-induced lymphocyte stimulation in patients with Hodgkin's disease was published in May. It was found that there was no correlation between PHA-induced lymphocyte transformation at the time of diagnosis and the clinical course of the patient's Hodgkin's disease. There is also no correlation between percent transformation and the histologic pattern of nocular sclerosis. Although PHA-induced lymphocyte transformation appears to be a general index of immunological status, it has proven to be of no value as a prognostic sign in Hodgkin's disease.

Because the nonspecific tests of general immunologic status have not proven helpful in prognosis, current studies are now under way investigating EBV titers as well as tumor cell membrane extracts containing EBV associated and EBV negative antigens. A prospective study of

delayed hypersensitivity reactions to autologous tumor extracts will be undertaken in patients with Hodgkin's disease, Burkitt's tumor, and other lymphomas.

2. Tumor specific antigen in ovarian carcinoma

Associated with the recent clinical interest in ovarian cancer, samples of human ascites tumor have been grown in cell culture, and a program of antigenic extraction and animal immunization is underway to produce high titer specific antibody.

3. The etiology of the anergic state - the relationship of anergy to pyridoxal phosphate deficiency in man

Pyridoxal phosphate (PLP) is known to play a necessary role in the maintenance of normal immunologic responses in experimental animals. Its possible importance as a causative factor in human immunologic deficiency states has been suggested by previous studies from the Medicine Branch, which demonstrated a strong correlation between anergy and plasma PLP depletion in patients with Hodgkin's disease. Other studies have revealed a high incidence of lymphopenia, anergy, and altered tryptophan metabolism (a pyridoxal phosphate dependent process) in patients with other malignancies.

We have initiated an investigation of the effect of PLP depletion on the delayed hypersensitivity response of normal human volunteers. Subjects will be placed on a pyridoxine-deficient diet, following a thorough evaluation of their immunologic status with a battery of routine skin tests, plus the sensitizing hapten, DNCB. The induction of PLP depletion will be monitored by measurement of plasma PLP levels. At the end of 5 weeks, skin tests will be repeated, and PLP will be added to the diet. Skin tests will be repeated in 2 weeks. These observations are expected to clarify the possible role of PLP depletion on delayed hypersensitivity.

4. HL-A antigens in Hodgkin's disease

The HL-A leukocyte antigen system is the major histocompatability determinant in man. To date, over 25 different HL-A antigens have been identified. Such extreme polymorphism suggests that this system may have evolved as an immunologic surveillance mechanism against lethal oncogenic virus infections and somatic mutations. Specific immune responsiveness to certain synthetic antigens and susceptibility to some leukemogenic viruses are clearly related to the alleles of the major histocompatability locus in several animal species. Thus, searches for disease-HL-A antigen relationships in man seem warranted, although such association may not be as clear-cut as those in inbred animal strains.

Published reports differ widely on the frequency of certain HL-A antigens (A1, A3, A11, A5, W5, W10, W15, W18) in patients with Hodgkin's disease (HD). Preliminary evidence suggests that HL-A antigen frequency may be related to both the histologic pattern and prognosis in this disease. To date, we have compared the HL-A antigen frequency in 103 caucasian HD patients with 459 healthy caucasian controls. HL-A typing for A1, A2, A3, A9, A10, A11, A5, A7, A8, A12, A13 W5, W10, W14, W15, W17, W18, W19, W22, and W28 was performed by the standard lymphocyte microcytotoxic technique. Only HL-A5 was found to be increased in the combined patient population (frequency, 0.200 versus 0.060 for controls, $P<.01$). However, in our 39 patients with nocular sclerosis HD, HL-A5 did not differ from normal controls (frequency, 0.080 versus 0.060 for controls, $P>0.05$). The frequency of HL-A5 did remain significantly elevated in those patients with mixed cellularity (0.310), lymphocyte depletion (0.220), or lymphocytic predominance(0.330), compared to controls (0.060). Additional HL-A1 and W18 were significantly increased in mixed cellularity HD (0.510 and 0.130 versus 0.290 and 0.020 for controls, $P<.05$). HL-A5 was equally distributed among all the clinical stages in mixed cellularity but occurred infrequently in those patients with stages III and IV nodular sclerosis. Patients in the other histologic types were too few to allow comparison. No differences in distribution among clinical stages were noted for the other HL-A antigens. We found no differences in HL-A antigen frequency, as related to disease duration. When combinations of HL-A antigens were considered for patients and controls, no differences were observed. The genotypes of 49 patients were determined by HL-A typing their parents and siblings. A preferential genotype for HD was not detected.

These results suggest that disease susceptibility and subsequent histologic pattern may be influenced by factors related to the HL-A phenotype of the host. Discrepancies in some previous reports may, in part reflect different proportions of pathologic patterns in the sampled patient populations. A prospective study is necessary to confirm or disprove these observations.

Honors and Awards:

Dr. Graff

1. "HL-A antigens in Hodgkin's disease." Presented at the Annual Meeting of the American Association for Cancer Research, Boston, Massachusetts, May 1972.

Dr. Young

1. "Invasive pulmonary aspergillosis." Presented to the V. A. Lung Group, January 19, 1972.

2. "Infectious complications of malignancy." Presented at Grand Rounds, Letterman General Hospital, San Francisco, California, March 25, 1972.

3. "The immune profile in Hodgkin's disease." Presented at the Conference on Hodgkin's disease, St. Louis, Missouri, October 1971.

Publications:

1. Young, R. C., Corder, M. P., Haynes, H. A., and DeVita, V. T.: Delayed hypersensitivity in Hodgkin's disease. A study of 103 untreated patients. Amer. J. Med. 52:63-72, 1972.

2. Corder, M. P., Young, R. C., and DeVita, V. T.: Skin reactivity in patients with cancer. New Eng. J. Med. 285:483, 1971.

3. Corder, M. P., Young, R. C., Brown, R. S., and DeVita, V. T.: Phytohemagglutinin-induced lymphocyte transformation: The relationship to prognosis in Hodgkin's disease. Blood May 1972.

4. Young, R. C., Corder, M. P., Berard, C. W., and DeVita, V. T.: Delayed hypersensitivty and lymphocyte transformation in Hodgkin's disease: The effect on course and survival. Arch. Int. Med. (in press).

5. Young, R. C., Jennings, A., Bennett, J. E.: Species identification of invasive aspergillosis in man. Amer. J. Clin. Path. (in press).

6. Levine, A. S., Graw, R. G., Young, R. C.: Management of infections in patients with leukemia and lymphoma. Current Concepts and Experimental Approaches. Seminars in Hemat. April 1972.

7. Robbins, J., and DeVita, V. T.: Pneumocystis carinii pneumonia: Diagnosis and treatment. Ann. Thor. Surg. (in press).

Serial No. NCI-3405
1. Medicine Branch
(formerly Solid Tumor Service)
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chronic Myelogenous Leukemia

Previous Serial Number: Same

Principle Investigators: George P. Canellos, Vincent T. DeVita, Jacqueline Whang

Cooperating Units: Surgery Branch, NCI; Human tumor Cell Biology Branch, NCI

Man Years:

Total:	1.0
Professional:	0.85
Other:	0.15

Project Description:

This disease has been the subject of investigation in this unit for some years. This is a neoplasm which is of great interest to a medical oncology unit because:

(1) It is a disease whose cells can be studied chronically.

(2) Its evolution to a more malignant phase mirrors the natural history of many other human tumors. The evolution from hyperplasia to true neoplasia is an ideal model for biochemical studies of the mechanisms of differentiation.

(3) Chromosomal studies can be conducted serially.

(4) Leukocytes can be harvested for preservation studies and subsequent transfusion.

Although the chronic phase can be controlled by chemotherapy, the blastic transformation has been an extremely difficult phase of the disease to control and represents a challenge similar to acute myelogenous leukemia. Increasing efforts in tissue typing and blood cell preservation offer a unique opportunity in this disease, since it is one where the likelihood of developing acute leukemia within 5 years of diagnosis is at least 50-60 percent. It is attractive to postulate that hyperplastic marrow could be banked and reutilized at the time of blastic crisis, if chemotherapy failed to remit the disease. Such a program is in progress.

A. Cytogenetic correlations

The Philadelphia chromosome (Ph') marker is well-known to occur in 90 percent of the patients with this disease. We have been able to correlate accelerated form of the illness in about 60 percent of the cases with the appearance of additional chromosomal abnormalities. In addition, we are currently assembling a completed report on the clinical characteristics of 20 patients with CGL, but in whom no Ph' chromosome could be detected. It is of note that patients fall into two groups: i.e., very short survival, less than Ph+ CGL. or extremely prolonged, well in excess of the Ph+ average.

B. Therapeutic studies

1. Chronic phase

The chemotherapy of the benign phase of the disease has been confined to control of the proliferative aspects of the disorder, without attempts to treat presumptive blastic transformation. This is due to the fact that chemotherapy of blastic transformation has been beneficial to too few patients for a short period of time. A comparative study between busulphan and dibromomannitol revealed no significant difference between them. Since a variety of other medications, including antimetabolites, other alkylating agents, and splenic radiation, have been compared unfavorably with busulphan, other treatment modalities are best compared to the latter drug.

2. Splenectomy

Splenectomy is currently under investigation for (a) prophylactic treatment during the chronic phase of the disease, and (b) treatment of thrombopenia without evidence of blastic crisis. Eighteen patients have had a prophylactic splenectomy with no other alteration in mode of the treatment. Too few patients have entered blastic crisis to evaluate its role. Five patients have entered blastic crisis, and three have achieved a partial or complete chemotherapeutic remission. It is clear to clinicians caring for these patients that it facilitates their care to have had the organ removed. Seven out of of seven patients with prolonged thrombopenia in the chronic phase, preventing adequate treatment, were benefited by splenectomy, returning platelets to a normal level.

3. Blastic phase

We have completed a 30-patient study of the effect of vincristine and prednisone in the treatment of blastic crisis of CGL. Nine patients achieved complete and partial remissions with a significant prolongation of life in the responders. Cytogenetic studies revealed that aneuploidy, especially hypodiploidy, favored a response to this regimen.

Since July 1971, a randomized study between vincristine and prednisone, and cytosine arabinoside and 6-Thioguanine has been carried out. Remissions have been seen with both regimens, suggesting that a combination of all four drugs may be a future trial. It will be of interest to compare the results of these trials in patients with or without splenectomy.

4. Bone marrow transplantations

In HL-A and mixed lymphocyte reaction compatible situations, allogenic transplantation will be performed in select cases of the blastic phase of the disease in the coming year.

C. Laboratory Studies

1. Stem cell function

The importance of assays of stem cell function has been emphasized. We are currently evaluating studies of proliferation and functional integrity of fresh and frozen cells by their responsiveness to erythropoietin in vitro and growth in semisolid mediuam. Erythroid cells in CGL retain their responsiveness to erythropoietin. Similarly, granulocytic precurosrs appear to grow adequately in the culture when studied as fresh cells.

Honors and Awards:

Dr. Canellos

1. "CGL." Presented at the Educational Session of the American Society of Hematology, San Francisco, December 1971.

2. "Treatment of CGL." Presented at the Orange County Medical Association, December 1971.

3. "Treatment of chronic leukemias." Presented at the Raleigh Academy of Medicine, Raleigh, North Carolina, October 1971.

4. "The leukemias." Presented at Georgetown University School of Medicine, February 1972.

Publications:

1. Hakami, N., Neiman, P. E., Canellos, G. P., and Lazerseon, J.: Neonatal megaloblastic anemia due to inherited transcobalamin II deficiency. New Eng. J. Med. 285:1163, 1971.

2. Canellos, G. P., Nordland, J., and Carbone, P. P.: Splenectomy for thrombocytopenia in chronic granulocytic leukemia. Cancer 29:660, 1971.

3. Canellos, G. P., DeVita,V. T., Whang-Peng, J., and Carbone, P. P.: Hematologic and cytogenetic remission of blastic transformation in chronic granulocytic leukemia. Blood 38:671, 1971.

4. Canellos, G. P., Brown, C. N., and Schnipper, L. E.: Prolonged hematologic and cytogenetic remission in blastic transformation. Cancer (in press).

Serial No. NCI-3420
1. Medicine Branch
(formerly Solid Tumor Service)
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cellular Biology and Kinetics of Cellular Proliferation

Previous Serial No.: 6075

Principle Investigators: Vincent T. DeVita, Robert C. Young, George P. Canellos, Philip S. Schein, Bruce A. Chabner

Other Investigators: Richard Adamson, Charles Bagley, Vincent T. Oliverio

Cooperating Units: Laboratory of Chemical Pharmacology, NCI

Man Years:

Medicine Branch:	Total:	2.20
	Professional:	1.95
	Other: 0.25	0.25

Project Description:

Kinetics of cellular proliferation: Information derived from animal tumor systems has clearly shown that interrelating knowledge of the biology of tumor growth (cell kinetics) with therapy improved therapeutic responses.

The goals of the tumor cell kinetic program of the Medicine Branch have been:

1. To examine and develop methodology that might be useful in obtaining cell kinetics information in individual patients in a time period that may allow application of these data to the patient's therapy.

2. To describe the kinetics of human tumors, compare them to animal models, and to compare results to normal tissues, to the same tumor in different patients, and to different metastatic sites of the same tumor in the same patient.

3. To examine the manner in which cell cycle characteristics are changed in response to exposure to drugs.

4. To approach the problem of manipulating tumor growth characteristics in such a way as to increase its susceptibility to chemotherapeutic agents.

RESULTS

A. Animal Studies

1. Cell cycle effects of the nitrosoureas

Continued interest in the unusual kinetic effects of the nitrosoureas has been the source of a series of studies using both BCNU and, more recently, MeCCNU. BCNU, when administered in subcurative doses, alters the kinetics of the L1210 leukemia population, and recent studies have shown that transplants of these cells have persistent alterations in cell cycle. A percent labeled mitosis curve obtained 21 generations later had a different configuration than the control curve and demonstrated a prolongation of T_S and T_G. The configuration suggested that treatment with BCNU may have led to the selective growth of a population of cells with a longer T_G and less variation around the mean T_G.

Kinetic studies with MeCCNU have demonstrated that a prolongation of the S phase occurs shortly after exposure to MeCCNU, and this prolongation is significnatly different from control values, with as little as 12.5 percent of the optimum tumor dose for a single injection of the drug. The maximum prolongation is approximately three times the normal duration of DNA synthesis. This alteration in DNA synthesis persisted for 4 hours after the dose. In addition, growth fraction studies have indicated no persistent alteration in the growth fraction of cells regrowing after MeCCNU. Persistent alteration of the generation time occurs after exposure to MeCCNU. The prolongation of generation time would seem to result from a selection out of cells with shorter cell cycle times.

Similar kinetic studies are now underway with procarbazine and methotrexate.

2. Simultaneous kinetic studies in normal and tumorous tissues using labeled precursor incorporation into DNA

Studies have been underway this year using a new technique of the simultaneous kinetic study of tumor tissue, normal bone marrow, and gastrointestinal mucosa using tritium labeled thymidine or deoxyuridine incorporation into DNA. Drug therapy can then be introduced and the effect of therapy on the kinetics of both normal and tumorous tissue can be studied over a period of time. Using an L1210 leukemia system and cytosine arabinoside, we have been able to show a differential recovery time between the tumor and normal tissue and, using scheduling which takes advantage of this difference, have been able to design a therapeutic regimen which results in prolonged survival with no increase in toxicity to normal tissues. Similar studies have been completed

with MeCCNU and with methotrexate, and are underway with 5-FU and Actinomycin-D. These techniques offer the possibility of developing scheduling of chemotherapeutic agents by a design based on knowledge of differential kinetics in the tumor and normal tissues.

3. Correlation of Methotrexate blood levels with kinetic effect on normal and tumorous tissues in vivo

In cancer chemotherapy, drug concentrations in serum x the duration of the effective level (G X T) has been proposed as the primary determinant of the effect of a given drug dose on tumor, bone marrow, and other target tissue. However, this concept makes no allowance for the differing sensitivities of the various tissues to the agent in question. As an approach to this problem, the biochemical effects of methotrexate (MTX) on various tissues have been followed as a function of the drug in plasma concentration.

MTX specifically inhibits the conversion of deoxyuridine (UdR) to thymidine in mammalian cells. The time course of this inhibition has been studied in mice by determining the incorporation of UdR into DNA after MTX administration. For a wide range of doses, escape from inhibition of the incorporation of UdR into DNA was found to occur when plasma MTX had fallen to 10^{-8} M. Recovery took place more rapidly in bone marrow than in gastrointestinal mucosa or ascites tumo this correlates well with the observation that the intestinal mucosa is the primary target organ for lethal doses of MTX in mice. The presence of L1210 ascitic lymphoma did not alter the pharmacokinetics of MTX or the duration of its biochemical effect on bone marrow and intestinal mucosa. It is hoped that similar studies in humans will provide information as to the relationship of plasma MTX to bone marrow and tumor DNA synthesis.

4. Modification of normal marrow kinetics by hormonal agents

The ability of granulocytic precursors to grow in semisolid medium has been utilized to study the cytokinetic effects of androgens administered in vivo to C_{57}/B_1 mice. In addition, androgen rich plasma was studied in vitro. The cloning of precursors was enhanced almost two times by the previous administration of androgens. In order to study the mechanism, androgen-treated marrow was studied in H_3TdR, incorporation, and hydroxyurea suicide to determine whether the steroids increased the number of cells in S-phase. The results indicated that androgen-treated marrow cells had an increased sensitivity to DNA synthesis inhibition, which was not seen following corticosteroids. The effect of 5-βH steroids on the in vitro behavior of myeloid cells and mouse leukemic cells is under study.

a. The effect of androgenic steroids on the erythroid stem cell

The mechanism whereby androgens affect erythropoiesis is being investigated pari pasu with the granulopoietic effect. The in vitro modification of the cellular response to erythropoietin in stimulation of hemesynthesis is being investigated. In addition, studies to attempt cloning of erythroid colonies have been initiated.

b. Humoral control of granulopoiesis

A murine tumor (MACA 1640) has been associated with extensive granulocytosis. The plasma and tumor extracts of this tumor are under investigation as a source for a granulopoietic substance. Although extracts are active in vivo, the ability of this material or materials to support in vitro growth is limited, suggesting it to be different from colony stimulating factor. The presence of inhibitors is currently being researched.

B. Human studies

Conventional kinetic studies are difficult to carry out in man, but we have been able to do some of these studies in select patients, and the design and details of the studies were outlined in last year's Annual Report. Acute leukemia and blastic phase of chronic myelogenous leukemia have been studied by the in vivo double labeling technique with direct injection of isotope into the bone marrow. Over 15 patients have been studied with this approach.

More recently, interest has centered on the use of the technique of labeled precursor incorporation into DNA simultaneously studied in the bone marrow and ovarian ascites tumor in humans. One such study has been completed, and more are planned. Using tritiated deoxyuridine incorporation into DNA and methotrexate therapy, we have been able to chart the kinetic effect of this drug on normal bone marrow and on ascites tumor. Simultaneous studies of the blood and ascites levels of MTX have also been performed. Findings suggest that, at least in this one patient, the optimum scheduling would be an every 5 day course. This approach may enable us to finally get the information we need to properly design schedules of chemotherapy in man. Studies in man are being planned for MTX, 5-FU, and cytosine arabinoside in patients with ovarian tumor, acute leukemia, and CML in blast crisis, as well as other solid tumors accessible to repeated biopsy.

Honors and Awards:

Dr. Young:

1. "The kinetics of solid tumor growth." Presented at Walter Reed Army

Hospital, November 1971.

2. "Human Solid Tumor Kinetics." Presented at the Symposium on Surgical Cancer Therapy, NIH, December 2, 1971.

3. "Cell Kinetics and the Chemotherapy of Cancer." Presented to Combined Clinical Staff Conference, NIH, September 1971.

4. "The Kinetics of Human Solid Tumor Growth." Presented at Letterman General Hospital, San Francisco, March 22, 1972.

Dr. DeVita

1. "Tumor Cell Kinetics." Presented at the University of Washington, Department of Medicine, May 2, 1971.

2. "Cell Kinetics and the Chemotherapy of Cancer," and "Management of Hodgkin's disease." Presented at Loma Linda University, January 12, 1972.

3. "The Biology of Tumor Cell Growth." Presented at the Department of Pharmacology, George Washington University School of Medicine, March 6, 1972.

4. "Tumor Cell Kinetics." Presented to the Combined Clinical Staff Conference NIH, September 1971.

5. "Cell Kinetics and Cancer." Presented at the Sixth Annual Cancer Symposium Akron City Hospital, March 18-19, 1971.

Dr. Canellos

1. "The effect of Androgenic Steroids on Granulopoiesis." Presented at the 14th Annual American Society of Hematology, San Francisco, December 1971.

Publications:

1. DeVita, V. T.: Cell kinetics and the chemotherapy of cancer. Cancer Chemother. Rep. 2:23, 1971.

2. Muggia, F. M., DeVita, V. T.: In vivo tumor cell kinetic studies: Use of local thymidine injection followed by fine needle aspiration. J. Lab. and Clin. Med. (in press).

Serial No. NCI-6500
1. Medicine Branch
(formerly Solid Tumor Service)
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Physiologic Disposition, Metabolism, Secretion, and Interaction of Antitumor Agents

Previous Serial No.: None

Principle Investigators:

Medicine Branch: George P. Canellos
Bruce A. Chabner
Vincent T. DeVita
Philip Schein
James Arseneau
Robert C. Young
Charles M. Bagley

Laboratory of Chemical Pharmacology: Vincent Bono
Vincent Oliverio
David Johns
Robert Sponzo

Man Years:

Medicine Branch:	Total	3.25
	Professional:	3.1
	Other:	0.15

Project Description:

A. Methyl CCNU

A phase I evaluation of this agent is currently underway. Studies of the physiologic distribution of radioactive methyl CCNU [1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea] have been conducted in six patients receiving drug labeled with ^{14}C in either the cyclohexyl or ethylene moieties.

The drug was given orally in doses ranging from 120 mg/m^2 to 220 mg/m^2. Significant plasma levels of radioactivity were detected as early as 10 minutes with peak levels occurring at 3 to 6 hours. Plasma levels of isotope were prolonged with an average half-life of 33 hours. Approximately 60 percent of the radioactivity given was excreted in the urine within 36 hours, and of this more than 50 percent was excreted in the

first 12 hours. Cerebrospinal fluid samples obtained at 2 and 3 hours, respectively, from two patients given cyclohexyl-labeled MeCCNU showed concentrations which were only about 10 and 15 percent of concurrent plasm ^{14}C levels. Degradation is extremely rapid since parent compound was not detected in plasma samples as early as 10 minutes, or in CSF and urine samples.

B. Cyclophosphamide

Although the pharmacology and drug interaction of cyclophosphamide have been extensively studied in animals, there are no comprehensive studies of this widely used drug in humans.

Since May 1971, 35 doses of ^{14}C cyclophosphamide have been given to 21 patients with various malignancies in drug doses of 10-80 mg/kg on various schedules, by intravenous route. Blood isotope and free cyclophosphamide levels and drug disposition in expired air, urine, and feces have been measured by scintillation counting after appropriate extraction or combustion of samples. Free alkylating metabolite levels have been determined in plasma, ascites, and urine by reaction with gamma-(4 nitrobenzyl) pyridine. Biologic activity of plasma alkylating metabolites is being investigated by L1210 in vitro killing, in cooperation with Dr. Richard Adamson, Laboratory of Chemical Pharmacology, NCI.

Results to date show a significant correlation of alkylating levels with drug dose and with marrow toxicity. The half-life of free cyclophosphamide becomes more prolonged at higher dose levels, but the fraction of unmetabolized drug excreted in the urine does not vary with dosage. Expired air and stools contain only 1-3 percent of total label excreted. Renal disease appreciably prolongs alkylating metabolite plasma levels and may increase toxicity. Chronic administration of steroids, and possibly also barbiturates, induces more rapid drug metabolism. At present, the effects of acute administration of steroids and phenothiazines and chronic allopurinol therapy are being examined.

C. Streptozotocin

Preliminary studies have indicated that this new agent inhibits synthesis of the coenzyme NAD. Dr. Philip Schein is currently investigating the effect of streptozotocin on the NAD biosynthetic pathway in an attempt to identify the drug's site of action.

D. Carboxypeptidase G_1

Studies are in progress to define toxicity, immunogenicity, and antitumor activity of Carboxypeptidase G_1 ($CPDG_1$), a folate-cleaving enzyme isolated from Pseudomonas stutzeri. Complete inhibition of a variety of murine

tumor cell lines in vitro resulted from exposure to 0.025 units/ml of $CPDG_1$. The cytotoxic effects of $CPDG_1$ in culture, as measured by soft agar cloning, were apparent after 6 hours exposure to the agent, while inhibition of DNA synthesis occurred within 1 hour of exposure. $CPDG_1$, 800 units/kg qd x 5, significantly prolonged survival of mice inoculated with L1210, L5178Y, and sarcoma 180. However, the most marked antitumor effect was observed in rats with Walker 256 carcinosarcoma, in which an increase of life-span of 43-100 percent was observed.

No pathologic or histologic changes were detected in mice which had received 800 units/day for 11 days. Mice tolerated up to 5000 units in a single dose with minimal weight loss and no morbidity. No antibodies were detected following acute or chronic administration. A sensitive radioimmunoassay for anti-$CPDG_1$ antibodies has detected anti-$CPDG_1$ antibodies in rabbits injected with $CPDG_1$ in Freund's adjuvant, but no antibody in mice treated daily for 11 days.

The Drug Research and Development Section of Experimental Therapeutics, NCI, has initiated arrangements for purifying large quantities of $CPDG_1$ for patient studies. In conjunction with these plans, studies are in progress to simplify the purification of the enzyme using cationic exchange resisn or affinity chromatography. These studies have shown that the enzyme adheres to SP-Sephadex at pH 5.1 and is eluted by an increase in molarity from 0.05 to 0.4; this procedure may be substituted for Sephadex G-150 chromatography or electrofocussing in the final purification scheme.

Finally, the in vivo hydrolysis of methotrexate (MTX) by $CPDG_1$ has been under investigation. The toxicity of high doses of MTX results from the prolonged persistence of MTX in plasma. $CPDG_1$, given intraperitoneally, was capable of hydrolyzing MTX in plasma, and when administered 24 hours following MTX, prevented the lethal toxicity of high doses of MTX. The use of otherwise lethal doses of MTX (350-1000 mg/kg) in this manner has led to a greater increase in the life span of tumor-bearing animals than possible with conventional single-dose therapy. A comparison of $CPDG_1$ and leucovorin rescue is in progress.

E. Gallium Nitrate

The demonstration that the isotope of Gallium (Ga^{67}) can be taken up by tumor cells as well as substantial animal tumor studies, which indicate that the salt possesses antitumor activity, has prompted a clinical pharmacologic investigation. The methods have included: (1) physiologic disposition of Ga^{67} in man; (2) radioautography of leukemic cells; (3) in vitro uptake study of sensitive and resistant animal tumor cells; and (4) cell separation techniques to define localization as to species.

In vivo and in vitro studies with ^{67}Gallium citrate in animals and humans are in progress.

With Dr. Roger Aamadt of the Department of Nuclear Medicine, retention studies of ^{67}Gallium citrate, injected for whole body scans, are being performed. Serial whole body counts are correlated with serial blood, plasma, urine, and fecal levels. Whenever possible, biopsy material obtained during the study is being autoradiographed to determine cellular localization of gallium. Interesting data thus far concern the propensity of elukocytes to concentrate gallium to an extent which, in a patient with a high WBC count, can actually alter the whole body excretion pattern. Cell separated studies with Dr. Warren Evans suggest, thus far, that mature polymorphonuclear leukocytes have the greatest ability to concentrate ^{67}Ga. Radioautographic confirmation studies are in progress.

In vitro studies with L1210 and Walker cell lines suggest that Gallium uptake may be related to phagocytosis and/or pinocytosis and that nonspecific stimulation of either of the above may greatly enhance ^{67}Ga uptake. A consistent difference in uptake between L1210 (sensitive to Ga in vitro) and Walker (resistant to Ga in vitro) was not demonstrated.

Of clinical significance is the ability of ^{67}Ga to localize abscesses and soft tissue aggregation of CML leukocytes on scan. The relation of the scan findings to our theory of phagocytosis-pinocytosis as the chief method of ^{67}Ga uptake is being explored

F. Procarbazine

Drug distribution and metabolism studies are in progress in conjunction with the Phase I clinical trials of the methylhydrazine derivative, procarbazine. Evidence indicates that the intact parent compound is oxidatively altered to first an active then an inactive metabolite, raising the potential for alteration of toxicity or clinical effect by drugs known to stimulate or inhibit oxidative metabolism.

Honors and Awards

Dr. Chabner:

1. "Prevention of MTX toxicity by carboxypeptidase G_1." Presented at the American Association for Cancer Research, Boston, Massachusetts, May 5, 1972.

2. "Immunological and immunochemical studies on carboxypeptidase G_1 (CPD G_1), a folate-cleaving enzyme. Presented at the Federation meetings, April 11, 1972.

3. "Carboxypeptidase G_1 (G_1): A new approach to antifolate chemotherapy." Presented at the American Cancer Society's Seminar for Science Writers, March 24-29, 1972.

Publications

1. McCullough, J., Chabner, B. A., Bertino, J. R.: Purification and properties of carboxypeptidase G_1. J. Bio. Chem. 246:7207-7213, 1971.

2. Chabner, B. A., Bertino, J. R.: Activation and inhibition of carboxypeptidase by divalent cations. Biochem. and Biophys. Acta (in press).

3. Chabner, B. A., Chello, P. L., Bertino, J. R.: Antitumor properties of carboxypeptidase G_1, a folate-cleaving enzyme. Cancer Res. (in press).

4. Haskell, C. M., Canellos, G. P., Hardesty, C., Cooney, D. A.: Clinical pharmacology of purified crystalline asparaginase. Cancer Chemother. Rep. (in press).

5. Canellos, G. P., Haskell, C. M., Brown, C. H.: Asparaginase synthetase in normal and leukemic leukocytes: A mechanism of cellular resistance. Colloques International 197:115, 1971.

6. Bagley, C. M., Bostick, F., DeVita, V. T.: Alkylating metabolites of cyclophosphamide. Proc. Amer. Assoc. Cancer Res. 13:99, 1972.

Serial No. NCI-3400
1. Hematology & Supportive Care Branch
2. Clinical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Chemotherapy of Acute Leukemia

Previous Serial Number: Same

Principal Investigators: Edward S. Henderson, M.D., Brigid G. Leventhal, M.D. Ronald A. Yankee, M.D., Arthur S. Levine, M.D., and Robert G. Graw, M.D.

Other Investigators: Roger H. Halterman, M.D., Geoffrey P. Herzig, M.D., Robert L. Carolla, M.D., Julian M. Duttera, M.D., Kenneth S. Graff, M.D., Herbert Kann, M.D., Harvey Gralnick, M.D., Jacqueline Whang-Peng, M.D., David Johns, M.D., Graham Smith, M.D., Joan Bull, M.D., and Paul P. Carbone, M.D.

Cooperating Units: Acute Leukemia Group B
Laboratory of Chemical Pharmacology, ET, NCI
Laboratory of Tumor Cell Biology, ET, NCI
Hematology Service, Clinical Pathology, CC, NCI

Man Years:

Total: 6 1/2*
Professional: 4 1/2*
Other: 2

*Exclusive of first-year Clinical Associates

Project Description:

Objectives:

1) To assess the effectiveness against acute leukemia of new drugs (5XX).

2) To improve the effectiveness and safety in leukemia treatment of compounds with known activity by improved scheduling and/or combination administration (6X1; 6X2; 7XX).

3) To relate clinical effects of drugs to clinical and pre-clinical pharmacology, activity, toxicity and model systems to improve both aspects of drug development (2AX, 2B1, 2B2, 3X1, 6X2).

4) Same

5) Same (7XX)

Major Findings:

I. New Drug Trials (Phase 2)

A) VM26 (epipodophyllotoxin-4'-Demethyl-9-(4,6-O-2-thenelidine-B-D glucopyranoside) (NSC-122819) This compound was administered to 14 patients (12 children and 2 adults) with refractory acute lymphoblastic leukemia (ALL) and 7 patients (5 adults and 2 children) with acute myeloblastic leukemia (AML). No complete remissions were noted in either group, however reduction in circulating blasts were noted in half the AML patients, and a partial remission was seen in one child of 15 with AML. The study is terminated in ALL, but will continue in AML to attempt to confirm and extend responses in this category.

B) Methyl CCNU (NSC-79037) The protocol was designed to complement ongoing phase 1 trials with this compound, dose levels in the phase 2 study following one dosage step behind those concurrently administered in phase 1. 8 patients with ALL have been entered at doses of 90-220 mg/m^2 q 6 weeks. No activity was noted at dosages less than 170 mg/m^2. One of two patients receiving 170 mg/m^2, an 8 year old girl with ALL, has achieved M_1 marrow status. The study will continue in both ALL and AML at dosages of 170-220 mg/m^2 a range at which toxicity should not be limiting and antileukemic effect would be anticipated.

C) Rifamycin SV (NSC-133100) A study has been instituted to evaluate this reverse transcriptase inhibitor in patients whose leukemic cells are shown to be sensitive in vitro to concentrations obtainable by parenteral administration. 4 children, 2 with AML, and 2 with ALL, have been studied to date. In each instance inhibition of DNA synthesis and/or cytolysis of the leukemic blasts in vitro occurred at concentrations of 50-100 mg/ml of Rifamycin SV. Plasma levels of 100 ug/ml or higher were obtainable in all four patients. No complete or partial remissions have been obtained to date. However a transient reduction in bone marrow lymphoblasts from 70% to 35% was noted in one patient. Toxicity was minimal except for persistent asymptomatic hyperbilirubinemia occurring in three of the four patients, which persisted several weeks after discontinuance of the drug. Despite this chemical abnormality this compound will be further assessed in both relapse and remission.

II. Drug Combinations (Childhood ALL)

A) New POMP. This program consisted of intensive four drug induction and maintenance schedule continued for six months into remission, then a pulse dose of BCNU followed by randomization between no further therapy and six additional monthly courses of the four drug combination. The

additional therapy resulted in significantly increased duration of remission (18) months versus 12 months, for those reaching the randomization point). Only one of 21 patients receiving six months therapy remains in remission, while 8 of 23 treated for 12 months are in continuous remissions of 2-4 years duration. The Life Table projection of survival of this entire protocol group of 66 children, responders and non-responders, approaches four years.

B) The current first line protocol for childhood ALL includes POMP induction and consolidation, central nervous system (CNS) irradiation plus intrathecal drug administration, and a comparison of immunotherapy with intermittent methotrexate at repetitive periods during remission maintenance (see Annual Report NCI-6302). To date 30 of 31 evaluable patients have achieved remission. Of these, four developed CNS leukemia prior to the CNS treatment phase. Only one of 21 receiving CNS therapy has subsequently suffered meningeal relapse. The earliest hematological relapse occurred four months into remission and at present 24 patients remain in complete remissions for periods up to ten months.

Childhood AML:

17 children with AML received remission induction with either POMP, PRAVD (prednisone, vincristine, cytosine arabinoside, daunorubicin), or alternating courses of each combination. Sixteen of the 17 (94%) achieved complete remission of 2 to 30+ months (median 11+ months). Median survival for the group is in excess of 21 months, or 150% of previous best results in this disease category in children.

Adult ALL:

In conjunction with Acute Leukemia Group B POMP was compared to the three drug combination of vincristine, prednisone, and daunorubicin. The induction rates for both regimens was 50%. Patients induced with POMP were maintained with intermittent courses, while patients induced with VDP received intermittent courses followed by intermittent injections of methotrexate with periodic reinduction with the inducer agents. The protocol was subsequently revised to include L-asparaginase during remission induction, consolidation, and maintenance. With this revision the VDP plus asparaginase arm exhibited superior remission induction and remission maintenance capabilities with all but one patient still in remission (the longest 460+ days to date).

Adult AML:

POMP vs PRAVD. Remissions lasting a median of 8.5 months were achieved in 37.5 percent of adults treated with this regimen. Cytosine arabinoside and 6-thioguanine. 100 mg/m^2 or each drug was administered every twelve hours to the point of tolerance. To date the remission rate has been 75 percent, encouraging the thought that in the future,

the major chemotherapy effect in this disease (as with ALL of childhood) should be directed toward remission maintenance. The current adult maintenance regimens compare cytosine arabinoside plus BCNU versus cytosine arabinoside, BCNU, and hydroxyurea.

This maintenance study was based on 1) the synergism noted in animals through sequential blockage of de novo pyrimidine synthesis (hydroxyurea) and nucleotide polymerase (cytosine arabinoside) and 2) studies of sequential treatment with BCNU and cytosine arabinoside which demonstrated less than additive stem cell toxicity through this schedule of administration. Clinical toxicity has been considerable but tolerable, and is not significantly increased through the use of BCNU. Median durations of remission are 4 months in both maintenance arms.

Treatment for Meningeal Relapse:

The effect of methotrexate and cytosine arabinoside were compared 21 patients suffering meningeal leukemia relapse. To date no significant difference in response has been used. The addition of 2400 rads of cranial radiation has not increased response rates, but the more important question of remission duration is not yet evaluable. Toxicity has been frequent and occasionally severe, in contrast to the acceptable toxicity in patients receiving drug plus irradiation early in remission, at a time at which meningeal leukemia was not detectable. Severe reactions appear more common in patients receiving methotrexate formulated with preservatives (para-Aminobenzoate derivatives).

III. Patient Survival

Survival from diagnosis has increased in all categories of childhood acute leukemia during the last 5 years. For example 3 year survival for childhood ALL was 39% in 1965-1967 compared to 55% from 1968-1970. Five year survival for ALL in 1965-1967 was 21%; 5 year figures are not yet available for patients admitted since 1967. As noted earlier median survival for childhood AML before 1970 was only 12 months compared to 21+ months in the 1970-1972 period. Survival has not increased for either ALL or AML in adults. The medians have ranged from 7 to 13 months in ALL and 4 to 8 months for AML.

IV. Significance

Combination chemotherapy remains the bulwark of treatment of childhood and adult acute leukemia of all morphological categories. As noted in reports of prior years major problems limiting success of treatment are 1) toxicities to bone marrow, liver, kidneys, and gastrointestinal tract 2) extramedullary leukemia cell infiltrations especially those of the central nervous system, and 3) failure of host immunological function to adequately curtail and/or eliminate post-chemotherapy residual disease.

New drugs continue to be an urgent requirement, particularly those grounded in precise knowledge of biochemical differences between normal and malignant tissue and further understanding of the regulatory mechanisms of cell division and cell differentiation. Cell membrane active agents and those directed against carcinoembryomic enzymes (e.g. reverse transcriptase) are of high priority for development and for clinical trials. Pre-treatment in vitro assays of drug efficacy should be incorporated whenever possible to reduce drug toxicity and increase effectiveness.

Although currently available methods for CNS sterilization are effective, they are not specific and accordingly are excessively toxic. Intensive antimicrobial prophylaxis and leukocyte transfusion support continue to be necessary adjuncts for leukemia chemotherapy, and exploration of native immunological defenses must be closely intercalated into drug therapy regimens.

V. Proposed Course of Project

1. Continue the search for optimal drug combinations, to include the use of new active agents (e.g. methyl CCNU) and agents with disparate mechanisms of actions and toxicities (e.g. VM26, rifamycin).

2. Develop in vitro assays for estimation of response to all major therapeutic modalities.

3. Continue development of alternate methods of CNS leukemia treatment and prophylaxis.

4. Utilize currently developing capabilities for animal and computer models of pharmakokinetics.

Honors and Awards:

Member, Section in Hematological and Oncological Drugs, U.S. Pharmacopaeia.

Member, Board of Medical Advisors, Leukemia Society of America, Washington Chapter.

Invited Lectures:

Dr. Edward S. Henderson

1) American Cancer Society, South Carolina Division, Murtle Beach, September 1971.

2) Raleigh Academy of Medicine. Symposium Chairman and Speaker, Raleigh, North Carolina, October 1971.

3) Guest editor, Symposium on recent advances in leukemia and lymphoma therapy, Seminars of Hematology in press April 1972.

Publications:

1) Goodell, B., Leventhal, B.G., and Henderson, E.S.: Cytosine arabinoside treatment of acute granulocytic leukemia. Clin. Pharmacol. Therap. 12: 599-606, 1971.

2) Goldin, A., Sandberg, J.S., Henderson, E.S., Newman, J.W., Frei, E., III, Holland, J.K.: The chemotherapy of human and animal leukemia. Cancer Chemo. Repos. 55: 309-507, 1971.

3) Preisler, H.D., and Henderson, E.S.: Effect of cytosine arabinoside and 1, 3-Bis(2-chloroethyl)-1-nitrosourea on hematopoietic precursors in the mouse. J. Nat., Cancer Inst. 47: 971, 1971.

4) Leventhal, B.G., and Henderson, E.S.: Therapy of acute leukemia with drug combinations which include asparaginase. Cancer 28: 825-829, 1971.

5) Leventhal, B. G. and Henderson, E.S.: Combination Therapy of Acute Leukemia with Asparaginase Plus Other Cytostatics. Colloques Internationaux CNRS No. 197--La L-Asparaginase (Eds.) Bernard, J., Boiron, M., Jacquillat, Cl., Weil, M. and Levy, D., Paris, Editions du Centre National de la Recherche Scientifique, 1971, pp. 286-294.

6) Duttera, M.J., Galleli, J.F., Kleinman, L.M., Langrea, J.A., and Wittgrove, A.C.: International methotrexate. Lancet 1: 540, 1972 (Letters to the editors).

7) Preisler, H.D., and Henderson, E.S.: Effect of suppression of erythropoiesis on hematopoietic stem cells in the mouse. J. Cell. Physiol. 79: 103-110, 1972.

8) Henderson, E.S.: Acute lymphoblastic leukemia. In Cancer Med., Frei, E., III and Holland, J.F., (eds.) Williams & Wilkin, 1972, (In press).

9) Henderson, E.S.: Introduction to the symposium on leukemia and lymphoma therapy. Seminars Hematol., 1972,(In press).

10) Duttera, M.J., Carolla, R.L., Gallelli, J.F., Gullion, D.S., Keim, D.E., and Henderson, E.S.: Hematuria and crystalluria after high dose 6-mercaptopurine administration. New Eng. J. Med., (In press).

Serial No. NCI-3407
1. Hematology and Supportive Care Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: 1) Studies on the Genetic Organization of the Small Oncogenic Papovavirus, SV40

2) Studies on *in vivo* Tumorigenesis and *in vitro* Transformation Induced by DNA Tumor Viruses: Viral and Host Components as Assayed by Biochemical and Biophysical Techniques

3) Studies on the Molecular Mechanisms by Which Human Herpes Viruses Establish a Latent State: The Passenger Situation, Recurrent Infection, and Putative Tumorigenesis

4) Studies on the Mechanism of Activation of RNA Tumor Viruses by IUDR and BUDR

Previous Serial Number: Same

Principal Investigator: Arthur S. Levine, M.D.

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Cooperating Units: Laboratory of Viral Diseases, NIAID: Lewis, Rowe, Kelly, Lowry, Chattymoluwi.

Research Division of Infectious Diseases, Boston Children's Hospital Medical Center and Harvard Medical School: Oxman, Levin.

Department of Medicine, University of Missouri: Henry, Samaha.

Man Years:

Total: 10
Professional: 9.5
Other: 0.5

Project Description:

Objectives:

1) To study the genetic organization of SV40 by an analysis of the varying segments of the SV40 genome contained within adenovirus-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical and EM techniques to arrive at a genetic map of an animal tumor virus.

2) To identify and isolate that gene(s) of SV40 which relate most directly to the virus' oncogenicity, employing the previously noted adenovirus-SV40 hybrid viruses.

3) To analyze the behavior of virus-transformed cells as a function of the molecular conformation of viral DNA within these cells, and as a function of events which may control viral genetic expression.

4) To analyze the behavior of permissive cells acutely infected by DNA viruses as a function of the molecular conformation of the viral nucleic acids and as a function of events which may control viral genetic expression.

5) To study the molecular mechanisms by which the human herpes viruses (H. Simplex, varicella-zoster, cytomegalovirus, and the Epstein-Barr virus) establish a latent state, as it relates to recurrent infection and to putative tumorigenicity. Such mechanisms will be approached with the techniques previously applied successfully to the smaller DNA tumor viruses.

6) To analyze the mechanism by which C-type murine leukemia virus is activated by the thymidine analogs, 5-iododeoxyuridine (IUdR) and 5-bromodeoxyuridine (BUdR).

Methods Employed:

Standard biochemical and radioisotopic methods have been employed for the measurement of nucleic acid and protein synthesis. Procedures for the extraction of highly purified viral and cell nucleic acids have been further improved. New techniques for hybridization of viral and cellular nucleic acids have been developed, and modifications which permit more quantitative comparison of heterologous nucleotide sequences have been described. Viral DNA has been analyzed by equilibrium density centrifugation (neutral and alkaline), analytical ultracentrifugation, ethidium bromide banding of superhelical DNA, temperature-absorbance profiles, and electron microscopic techniques. Standard tissue culture techniques have been carried out, as well as plaque assays and immunoflourescent assays for viral antigens. Localization of SV40 genes in adenovirus-SV40 hybrid DNA molecules has been accomplished by electron microscopic examination of heteroduplexes formed

with one strand of wild-type adenovirus DNA and one strand of hybrid virus DNA. In the past year, we have modified standard techniques of auto-radiography to allow sensitive in situ nucleic acid hybridizations. Methylated albumin kieselguhr (MAK) chromatography has also been employed to permit separation of differing species of DNA. Methods for separation of the strands of adenovirus DNA, using poly IG and isopycnic density separation, have been improved.

Procedures for fractionating the chromosomes of virus-transformed cells according to group-size have been developed, as have hybridization methods to determine which chromosomes contain integrated viral genes (using in vitro synthesized complimentary RNA).

Major Findings:

1. We have previously described the isolation and initial characterization of a nondefective adenovirus 2-SV40 hybrid virus (Ad_2+ND_1) which has proved exceedingly useful for biological and genetic studies of the small tumor virus SV40, since the hybrid replicates without any helper particles and contains a limited segment of the SV40 genome. Ad_2+ND_1 is equivalent to a self-replicating deletion mutant of SV40, containing approximately 250,000 daltons of SV40 DNA covalently linked to virtually all of the adenovirus 2 genome. This amount of SV40 DNA is sufficient to code for one average, or perhaps 2 small proteins. In fact, we have identified one SV40 antigen in cells infected by ND_1, the heat-stable SV40 "U" antigen, which is synthesized early in the SV40 lytic cycle, prior to viral DNA replication. In the past year, the SV40 moiety in Ad_2+ND_1 has been compared with wild-type SV40 virus in terms of sequence homology of the SV40-specific RNAs induced in cells infected by the respective viruses. By the technique of hybridization-competition, it was found that ND_1 induces about 1/3 of the RNA species transcribed early in the course of SV40 infection, and that early SV40 RNA is transcribed from about 1/3 of the total number of SV40 genes (8-10). Thus, the ND_1 virus contains one small segment of the SV40 genome; from this DNA is transcribed one species of SV40 early RNA, which directs the synthesis of one detectable early SV40 protein. Since most of the virus-specific RNA transcribed in virus transformed cells is early RNA, the utility of a virus which appears to contain one early SV40 gene is apparent.

Recently, 4 new nondefective adenovirus 2-SV40 hybrid viruses have been isolated and characterized, each inducing differing SV40 functions in acutely infected cells. $Ad.2+ND_2$ induces 2 SV40 antigens, U and TSTA (the tumor-specific transplantation antigen); ND_3 induces no SV40 antigens; ND_4 induces all 3 of the SV40 antigens detectable early in wild-type SV40 infection; U, TSTA, and T; and ND_5 induces no detectable SV40 antigens. (ND_3 and ND_5, originally thought to be non-hybrid Ad $_2$, were identified as hybrids by virtue of the fact that they do induce SV40-specific RNA in infected cells.)

2. We have now completed our investigation of the SV40 DNA and RNA sequences

associated with all 5 of the nondefective hybrids. Each hybrid DNA has a G + C % of 55-56, as does wild-type adenovirus 2. Length of DNA by electron microscopy is 10-11 μM, corresponding to a molecular weight of 20-21 x 10^6 daltons, similar also to adenovirus 2. Molecular weight of the hybrids by sedimentation in the analytical ultracentrifuge (S_{20W}) is approximately 25 x 10^6, also similar to wild-type adenovirus 2. All of the hybrid genomes contain covalently linked SV40 and adenovirus DNA sequences, as determined by alkaline sucrose rate-zonal sedimentation. The amount of SV40 DNA in each of the hybrids has been determined by reconstruction-hybridization, using in vitro synthesized complimentary SV40 RNA as a probe. The percentages of SV40 DNA in each hybrid genome are: ND_1, 1.0%; ND_2, 2.6%; ND_3, 0.2%; ND_4, 3.5%; and ND_5, 2.2%. These percentages correspond to the following molecular weights for the SV40 pieces: ND_1, 2.5 x 10^5; ND_2, 6.5 x 10^5; ND_3, 5 x 10^4; ND_4, 9 x 10^5; and ND_5, 5.5 x 10^5 daltons (wild-type SV40 DNA = 3 x 10^6).

3. The SV40 RNA species induced in infected cells by the 5 nondefective hybrids were compared by hybridization-competition. Results indicated that the similarity and diversity of SV40 RNA transcripts from the hybrids were in proportion to the percent of SV40 DNA in the various genomes. For example, ND_1 induced 28% of the SV40 RNA sequences induced by ND_4; ND_2, 70%; ND_3, 7%; ND_4, 100%; and ND_5, 55%. Therefore, none of the hybrid genomes contain redundant SV40 sequences, nor sequences which are not transcribed in vivo. The SV40 pieces in the 5 hybrid genomes must overlap one another with respect to their nucleotide sequence identity, with the shorter SV40 nucleotide segments entirely represented in the larger ones. We have also determined that all, or virtually all, of the SV40-specific information in the ND_4 hybrid is "early" SV40 information.

The foregoing data indicates an excellent correlation between the number of detectable SV40 functions induced by the 5 nondefective hybrid viruses, the size of their SV40 piece, and the number of discrete SV40 RNA species transcribed from their DNA. ND_1, with about one gene's worth of SV40 DNA, induces one SV40 protein in infected cells. ND_2, with 2 1/2 times as much SV40 DNA as ND_1, induces 2 SV40 proteins. ND_4, with 3.5 times as much SV40 as ND_1, appears to code for 3 antigens. All of these antigens are "early" ones (ND_1 = U; ND_2 = U, TSTA; ND_4 = U, TSTA, T), as is the SV40-specific RNA induced by each of the hybrids. The 3 early antigens are identical with those induced early (prior to viral DNA replication) by wild-type SV40, and also identical to those detectable in most SV40-transformed cells. It is likely that the ND_3 virus contains too little SV40 information to code for an SV40 peptide, but ND_5, containing more SV40 DNA than ND_1, remains enigmatic: possibly, a translational control is missing in this viral genome.

4. One model which might be proposed for the origin of these hybrids is this: in one intermolecular recombination, during mixed adeno and SV40 infection, the entire SV40 genome is inserted into linear adeno DNA. In a second recombination, varying lengths of the SV40 are deleted. With this model, SV40 is integrated as a single linear piece. Our data suggests that

the U and TSTA functions on the SV40 DNA segment within the ND_2 genome are adjacent. Thus, there are two possible arrangements for the three early functions on the segment of SV40 in ND_4: U, TSTA, T; or, T, U, TSTA. Electron microscopic heteroduplex mapping has been employed to visualize the SV40 pieces in the 5 hybrids, and to specify their anatomic linkage in relation to wild-type SV40. In all of the hybrids, the SV40 insertion site is about 14% from one end of the molecule, the SV40 DNA pieces all overlap at one end, and the opposite end varies in length. These findings make the former arrangement for the 3 early genes of SV40 highly probable, i.e., U, TSTA, and T in linear order. Consequently, the isolation of these 5 nondefective hybrid viruses has now permitted the genetic mapping of the early genes of SV40, i.e., those which appear to function in transformed cells.

5. Studies have also been completed on two variants of a defective adenovirus 2-SV40 hybrid population, Ad.2++ HEY and Ad.2++ LEY. These viruses are of interest because they each contain the entire wild-type SV40 genome and yield infectious SV40 virus in hybrid-infected cells; however, the two variants yield infectious virus with a 4-log difference in efficiency (HEY = high efficiency yielder, LEY = low efficiency yielder). We have sought to determine the molecular mechanism for this great difference in yielding efficiency. In hybridization-competition experiments we have found that the HEY virus induces SV40 RNA identical with that found in a typical wild-type SV40 lytic infection. LEY virus also induces RNA identical with the wild-type virus, but the slope of its competition against wild-type RNA indicates that a small number of SV40 genes are transcribed efficiently, and the remainder with marked inefficiency. It is likely from this and other data that with the LEY hybrid, the early genes are transcribed as in wild-type infection, but that the late genes are rarely transcribed. This might occur if the SV40 genome had to be excised from its integrated state in order for late genes to be transcribed. We have also examined each variant in the electron microscope (heteroduplex mapping) and find that there is somewhat more SV40 DNA in the HEY virus than the LEY. The "extra" DNA might be terminally redundant or in some other way permit more frequent excision of the SV40 genome from the HEY hybrid. This possibility is being explored. The importance of this HEY-LEY system lies in the fact that the configuration of SV40 and adeno DNA within these hybrids may bear upon the relation of SV40 to host DNA in SV40-transformed cells; thus, the system might provide a clue as to which mechanisms are necessary to permit a tumor virus genome to be excised from its integrated state (excision presumably being associated with viral replication and cell lysis).

6. The rapidly hybridizing RNA sequences transcribed in primary hamster embryo cells and in lines of serially propagated hamster cells were compared by the technique of RNA-DNA hybridization-competition. Such sequences are known to be transcribed from reiterated DNA sites. There were no rapidly hybridizing RNA sequences in primary hamster embryo cells which were not also present in transformed cell lines. However, in reciprocal experiments, unlabeled RNA from primary hamster embryo cells competed with only 62 to 66% of the hybridizable radioactivity present in RNA from transformed lines. In

contrast, the rapidly hybridizing RNA sequences detected in untransformed, contact-inhibited cell lines were similar to those present in transformed cells. Thus, primary hamster embryo cells appear to lack rapidly hybridizing RNA sequences that are transcribed in both transformed and untransformed cell lines. Moreover, these results indicate that the acquisition of oncogenicity is not necessarily correlated with the transcription of additional rapidly hybridizing RNA sequences, since such new sequences may already be present in serially propagated untransformed cells.

7. The virus-specific RNA and DNA of 2 clones of SV40-transformed hamster heart cells differing in oncogenic potential were studied by nucleic acid hybridization techniques. Reconstruction experiments using in vitro SV40 RNA as a probe demonstrated 2.0 viral genome-equivalents per cell in the clone having high oncogenic potential and 1.3 viral genome-equivalents per cell in the clone with low oncogenic potential. Cells of the low oncogenicity clone were found to synthesize less than one-fourth as much SV40-specific RNA as the high oncogenicity clone, but no difference in the species of SV40-specific RNA induced in the 2 clones was apparent. These results indicate that expression of the virus genome is qualitatively similar in cloned cells of high and low oncogenic potential, making it likely that variation in this potential is independent of the virus genome. However, the possibility cannot be excluded that small quantitative differences in the virus-specific nucleic acids of transformed cells are associated with altered oncogenicity.

8. We have begun to examine the mechanism(s) by which IUdR and BUdR activate C-type murine leukemia virus from cells which contain no detectable viral antigens or virus. This study utilizes AKR clones which activate readily and others which activate poorly. We have found that both types of cell incorporate the same amount of tritiated IUdR, using ^{33}P as a marker for the rate of DNA synthesis. Hence, the difference in activation is not dependent on differences in the rate of IUdR incorporation into DNA.

Proposed Course:

1. We have completed initial experiments that have as their goal a functional analysis of the varying SV40 genes contained within the 5 nondefective hybrid viruses. In experiments with two cell types, we have found that the early SV40 functions in which we have interest are also expressed by the early adenovirus genes to which the SV40 genes are covalently linked, e.g., in hamster cells, both the SV40 moiety and the adeno moiety appear to induce transformation and the induction of cellular DNA synthesis. Consequently, we plan to examine these functions in other cell types which may restrict adeno expression and permit SV40 expression. We shall also begin experiments to determine if the SV40 and adeno moieties of the hybrid genomes can be physically separated or differentially inactivated, with the SV40 retaining biological function. An attempt will be made to learn how the function of the varying SV40 genetic segments is controlled by the contiguous adenovirus genes. We will attempt to isolate the SV40 segments in the hybrid molecules and to introduce them in cell-free protein synthesizing systems so that their indi-

vidual products can be identified and compared with new proteins found in SV40-transformed or acutely infected cells, and those found in hybrid virus-infected cells. Attempts will be made to isolate and purify other non-defective adenovirus 2-SV40 hybrid viruses which may contain segments of the SV40 genome not present in the 5 viruses so far studied. The long-range goal of this work is to completely map an animal tumor virus genetically, to identify and isolate the gene(s) associated with oncogenicity, to learn what the product of this gene is, and what is the molecular mechanism of its activity.

2. We have improved methods for separating adenovirus DNA strands such that transcription can be localized to one or the other strand; these procedures will now be applied to the hybrid viruses so that the sense and nonsense strands of SV40 can be determined (SV40 strands are otherwise inseparable by bulk preparative techniques). Moreover, we shall determine if certain SV40 genes are transcribed from one strand and others from the opposite strand, and the direction of transcription in each of these situations. We shall also employ separated hybrid strands to determine if there exist areas of the SV40 genome which are not transcribed in vivo.

3. Experiments involving physical fractionation of chromosomes from tumor cells will continue, with identification of those chromosomes in which viral genes are integrated. When the system yields consistent results with in vitro hamster cells, it will be applied to human situations such as the trisomies, Fanconi's syndrome, and chronic myelocytic leukemia. These are situations in which malignancy is present or is highly likely to appear, and in which a high frequency of in vitro virus transformation can be demonstrated. It will be of interest to learn if a correlation can be made between chromosomal anomaly and location of viral genes. We shall also continue attempts to localize SV40 genes in specific host chromosomes by in situ (autoradiography) hybridization. The goal of this work is to determine which host chromosomal site is necessary and sufficient for transformation.

4. During the past year, we have been provided with Herpes simplex type II DNA under terms of a contract with Dr. Clyde Goodheart. We are in the process of determining if the herpes DNA is separable from any contaminating host DNA sequences when virus is purified by the newer methods in use in Dr. Goodheart's laboratory. If in fact, herpes viral DNA is entirely free of host DNA by sensitive assays, we shall begin a series of experiments to explore the molecular mechanisms involved in several situations.

Following are some of the situations which might be approached using purified herpes DNA, or complementary RNA generated in vitro from a pure herpes DNA template, as probes to detect the homologous viral nucleic acids in cells which otherwise give no indication of the viral presence. H. simplex, type 2, in human cervical carcinoma cells; EBV in non-producing Burkitt cells; H. samurai in New World monkey lymphoma and acute lymphocytic leukemia; H. simplex, type 1, in recurrent skin lesions (during latency); varicella-zoster during latency; and cytomegalovirus during latency (e.g., in blood

bank preparations). The initial approach in all these situations would be to learn if in fact the viral nucleic acids can be detected in the absence of any other morphological or biological evidence of the viral presence. If so, what are the molecular events which explain why a herpes virus remains latent as contrasted with the events occurring during frank infection; finally, what is the molecular basis for acute infection in one cell type by a herpes virus, as contrasted with tumorigenicity in a different cell type by the same virus.

5. We shall continue experiments to determine the mechanism of activation of the C-type murine leukemia viruses by IUdR and BUdR, having shown that high and low efficiency of activation does not depend upon the rate of IUdR incorporation into cell DNA. We are investigating several other mechanisms that might account for differences in activation; excision of an integrated viral genome, rate of transcription or translation of viral components or cell repressors/inducers of these viral components, and amounts of integrated/episomal viral DNA complementary to the tumor virus 70S RNA.

Honors and Awards:

1. Characterization of SV40 DNA Segments in the Nondefective Adeno 2-SV40 Hybrids, and of the RNA Species Transcribed from these Segments. Presented at the Tumor Virus Symposium, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1971 (Levine).

2. Isolation of Nondefective Ad2-SV40 Hybrids which Induce Differing SV40 Functions. Presented at the Tumor Virus Symposium, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1971 (Lewis).

3. Genetic Studies of SV40 using the Nondefective Adenovirus 2-SV40 Hybrid Viruses. Lecture presented at NIH, NIAID Laboratory of Viral Diseases, Nov. 1971 (Levine).

Publications:

1. Oxman, M. N., Levine, A. S., Crumpacker, C., Levin, M., Henry, P., and Lewis, A. M., Jr.: Studies of Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses. IV. Characterization of the Simian Virus 40 RNA Species Induced by Wild-type Simian Virus 40 and by the Nondefective Hybrid Virus, Ad. $2^{+}ND_1$. J. Virology 8: 215-224, 1971.

2. Henry, P. H., Levin, M. J., and Levine, A. S.: Composition of Lymphocytes and Plasma Cells (Chapter 97), and Metabolism of Lymphocytes and Plasma Cells (Chapter 98). In Principles of Hematology, Williams, W. J., Beutler, E., Craddock, C. G., Erslev, A. J., and Rundles, R. W. (Eds.). Blakiston, McGraw-Hill Publishers, 1972.

3. Levine, A. S., Oxman, M. N., Eliot, H., and Henry, P. H.: New Species of Hybridizable RNA in Contact-Inhibited as well as Transformed Hamster Cell Lines. Cancer Res. 32: 506-510, 1972.

Serial No. NCI-3415
1. Hematology & Supportive Care Branch
2. Clinical Oncology, Chemotherapy, NCI
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: NCI-IBM Blood Cell Separator and Leukocyte Transfusion Study

Previous Serial Number: Same

Principal Investigators: Robert G. Graw, Jr., M.D. and Geoffrey P. Herzig,M.D.

Other Investigators: Edward S. Henderson, Joan M. Bull, Shirley House, Carole Novick, Pamela Woodruff, Scott Volker, Larry Brinkman, James Hill, Frank Stout, P.J. Scullen, Richard Brooks, Eugene Rush, Ruth Estabrook, William Penland, Charles H. Kirkpatrick, Paul I. Terasaki, Michael Blaese, Richard Root, Joseph Buzak, John Nutter, Richard Simon, Richard Anderson, and Seymour Perry

Cooperating Units: Medicine Branch, NCI
Plasmapheresis Unit, NCI
Department of Surgery
Histocompatibility Typing Laboratory
Microbiological Associates Contract
Program Analysis Branch, NCI
Infectious Disease Section, NIAID
Division Research Services
Armed Forces Radiobiology Institute
Hazelton Laboratories
Microbiological Associates
ABCOR Corporation

Man Years:

Total:
Professional:
Other:

Project Description:

Objectives:

1) To continue development in the procurement of buffy coat collections in man utilizing the NCI-IBM continuous flow Blood Cell Separator and newer techniques.

2) To study the safety and efficacy of leukocyte replacement therapy in the supportive care of granulocytopenic immunodeficienct patients.

3) To determine the kinetics of a successful leukocyte transfusion response.

4) To study the role of histocompatibility typing with regard to leukocyte transfusions.

5) Evaluation of "leukoadhesion" as a method of granulocyte procurement for transfusion.

Methods Employed

1) The operation of the NCI continuous flow Blood Cell Separator (CFC) was continued with normal blood donors. Granulocytes were transfused into patients with documented bacterial septicemia, and local infections. Lymphocytes were collected for the preparation of transfer factor and other immunological studies.

2) Filtration leukapheresis (Leukopak®) was examined in greater detail for the procurement of granulocytes in large quantities. Investigations as to the biologic properties of cells collected with these techniques were made.

3) Leukocyte transfusion programs were continued in dogs in order to study the effect of prophylactic granulocyte transfusions in conjunction with preclinical bone marrow transplantation experiments.

4) In vivo collection studies (canine) were continued in conjunction with a contractor, ABCOR, in an attempt to improve leukocyte collection with available and modified Cell Separator equipment.

5) Regular data processing has been implemented for analysis of the leukocyte procurement and transfusion programs.

6) Preclinical canine models were devised to study the effect of supportive blood component transfusions (RBC, platelet and granulocytes) on animals given various doses of total body gamma irradiation. These same leukopenic animal models were used to study immune competence and peripheral blood phagocytic capacity of the altered host.

7) Canine models were used to study the kinetics of the immune response to transfused blood products (intravenous) as compared to immunity resulting following skin and corneal transplantation.

Major Findings:

1) Granulocyte (PMN) transfusion studies employing "compatible donors" has

continued to demonstrate that repetitive transfusions initiated as quickly as possible following the occurrence of gram negative bacterial septicemia significantly improves patient survival. One hundred percent of patients (1212) receiving four or more consecutive granulocyte transfusion completely cleared all evidence of infection without recurrence, while only 26 percent (37 patients) of patients in a concurrently treated control group (antibiotics alone without supportive leukocyte transfusions) survived. Studies in leukopenic dogs receiving similar doses of CFC granulocytes (per square meter of body surface area) have demonstrated qualitatively normal extravascular migration of transfused cells to skin windows even when the level of transfused PMNs have fallen 18 hours post transfusion to pre transfusion leukopenic levels corroborating the effectiveness of small numbers of transfused normal PMNs. Granulocyte increments in recipients of transfusions collected from CFC have now reached a level of 850/cu mm (corrected for a dose of 10^{10} PMNs transfused to a recipient with one square meter of body surface area). This post transfusion increment is compared to the median transfusion increment following cells collected by reversible leukoadhesion of 266 per cu mm one hour post transfusion. In order to more carefully delineate the role for granulocytes collected by this new technique, the majority of granulocytes for transfusions have been collected using this mode. Continued studies as to the biologic activity of cells collected with this method have demonstrated nearly normal in vitro bactericidal and phagocytic capacity. However, in vivo leukokinetic studies demonstrate abnormal intravascular circulation with primary localization in the spleen following transfusion accounting for a shortened intravascular survival (T 1/2 = 15 minutes) with a possible slow release of these initially hindered cells permitting a prolonged second phase of intravascular survival (T 1/2 = 15 hours). A majority of transfusions given utilizing Leukopak® collected cells have been associated with transfusion reactions not unlike those previously noted with leukoagglutinin positive immune reactions; however, these transfusion reactions are associated with the same level of intravascular increment post transfusion as those transfusions given without a transfusion reaction. Whether this associated transfusion reaction represents a significant deterrent for the use of these cells remains unanswered. Paired transfusions of CFC or "filtration leukapheresis" granulocytes into the same recipient from "compatible donors" have demonstrated a much better increment for the non-leukopak collected cells without associated transfusion reactions.

2) Canine leukocyte transfusions were utilized as an adjunct to preclinical marrow transplantation studies (primarily in an attempt to support animals through finite periods of granulocytopenia). The study reported last year using animals prepared with lethal doses of cyclophosphamide were continued and demonstrated that leukocyte transfusions by themselves could not be used to support animals following large doses of cyclophosphamide unless frozen autologous bone marrow was given to the animals 24 hours following the last dose of cyclophosphamide. "Marrow rescue studies" were initiated to study the possibility for destroying marrow allografts causing fatal graft-versus-host disease in the canine

model system. Please see project report NCI-6301). These studies have clearly demonstrated that animals suffering from graft-versus-host disease can have their transplanted bone marrow destroyed and frozen autologous bone marrow administered with success when repetitive PMN transfusions can be supplied to artificially maintain the phagocytic capacity of peripheral blood during the two weeks following the marrow ablative course of cyclophosphamide. These results have direct application and importance for human marrow transplantation studies where the question of adequate supportive care during ablation procedures has been raised. The ABCOR Corporation (contractor) has continued to study techniques to improve the in vivo procurement of granulocytes by CFC using dogs leukapheresed at another contractor in Bethesda (Microbiological Associates). They have made an in depth study of recirculation, elutriation and variations in bowl design which may alter the circulation dynamics of CFC. The results to date have been somewhat disappointing in that simple modifications to the current centrifuge bowl design have not significantly enhanced procurement efforts. An instrument has been designed as a biproduct of these in vivo analyses which will permit precise and reproducible collections of buffy coat using the current Cell Separator. Essentially the instrument consists of a photoelectric cell which can monitor the buffy coat output from the centrifuge and automatically control the placement of the red blood cell-plasma interface within the centrifuge bowl. This instrument will greatly simplify the operation of the Cell Separator.

3) Additional personnel hired for data processing capability have begun to enter past leukocyte transfusion data into a master transfusion file permitting analysis of factors important for a successful leukocyte transfusion response. This enhanced data processing capability should greatly improve our understanding of which factors may be important in patient survival.

4) Preclinical canine models have been used to study the role of the recipients' immune response against donor blood components as this immune response may modify subsequent transfusions. In vitro assays (lymphocytotoxicity, leukoagglutination, mixed leukocyte culture, lymphocyte cellular cytotoxicity, in vitro marrow colony culture) have been used to study in vivo sensitization following blood transfusions or skin grafting. These models have clearly demonstrated the kinetics of a normal immune response and will be subsequently used to monitor transfusions which will be given in association with immunosuppressive agents in attempt to block immunization, in association with transfusions given in a way to induce tolerance to donor antigens, and in attempt to document preformed and lasting immunity which would eventually interfer with successful blood component transfusion and bone marrow allografting. These preclinical experiments are vitally important to our better understanding of the variables determining a successful transfusion experience particularly in light of the complexities of modern day blood component therapy.

Proposed Course

1) Continued evaluation of better methods to procure granulocytes for transfusion. This will include a closer comparison of granulocytes collected by filtration leukapheresis and CFC. An attempt will be made to integrate the two techniques taking advantage of the "leukocytosis inducing factor" released during leukopak® procurement. Cells collected using both techniques will be intensively studied using conventional leukokinetic techniques in an attempt to better understand the function of transfused granulocytes in the altered host.

2) Peripheral blood leukocytes will be collected from patients with leukemia in remission in order to prepare large doses of transfer factor for administration as immunotherapy during remission.

3) Lymphocytes will be collected from normal donors with evidence of immunity to common infectious agents (virus and fungi) in order that transfer factor can be prepared and administered to patients with fevers of unknown origin. These studies will be performed in collaboration with investigators currently investigating the applications of transfer factor. Preclinical canine transfusion experiments will be continued in order to better understand the "immunization process" in an attempt to design protocols which will minimize in vivo sensitization or prevent sensitization to transfused blood products.

4) An expanded evaluation of typing of PMN antigens in donor-recipient pairs will be made in association with the current histocompatibility typing contract.

5) Continued investigation of new leukocyte procurement techniques in conjunction with the ABCOR Corporation.

Honors and Awards:

G. P. Herzig, R. K. Root, and R. G. Graw, Jr.:

1) Transfusion of granulocytes collected by continuous-flow-filtration leukapheresis, presented at the Proceedings of the 14th Annual Meeting of the American Society of Hematology, December 1971, p. 48.

Publications:

1) Siegel, S.E., Lunde, M.N., Gelderman, A.H., Halterman, R.H., Brown, J.A., Levine, A.S., and Graw, R.G., Jr.: Transmission of toxoplasmosis by leukocyte transfusion. Blood 37: 388-394, 1971.

2) Graw, R.G., Jr., Herzig, G.P., Eisel, R.J., and Perry, S.: Leukocyte and platelet collection with the continuous flow Blood Cell Separator from normal donors. Transfusion 11: 94-101, 1971.

3) Kirkpatrick, C.H., Rich, R.R., Graw, R.G., Jr., Smith, T.K., Mickenberg, I.D., and Rogentine, G.N.: Treatment of chronic mucocutaneous moniliasis by immunologic reconstitution. Clin.Exp.Immunol.9: 733-748, 1971.

4) Herzig, G.P., Root, R.K., and Graw, R.G., Jr.: Granulocyte collection by continuous-flow-filtration leukapheresis. Blood (In press).

5) Levine, A.S., Graw, R.G., Jr., and Young, R.: Infectious complications of leukemia and lymphoma. Seminars Hematol. (In press).

6) Graw, R.G., Jr., Herzig, G.P., Perry, S., and Henderson, E.S.: Normal granulocyte transfusions: Treatment of gram negative bacterial septicemia. Submitted for publication.

Serial No. NCI-3416
1. Hematology-Supportive Care Branch
2. Clinical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Infectious Complications of Malignancy: Natural History, Prevention, Diagnosis, and Therapy

Previous Serial Number: Same

Principal Investigator: A. S. Levine, M.D.

Other Investigators: S. Siegel, M.D., E. S. Henderson, M.D., J. Bennett, M. D. (NIAID,LCI), D. Henson, M.D. (NCI,LP), C. Patch, Ph.D., and J. Hauser, B.S.

Cooperating Units: Laboratory of Clinical Investigation, NIAID; Laboratory of Pathology, NCI; Hazelton Laboratories, TRW, Inc.; Infectious Laboratory Service, CP, CC; Nursing Service, CC; Central Sterile Supply, CC; Pharmaceutical Development Service, CC; Dietetics Service, CC; Environmental Sanitation, CC; Environmental Services, CC; Sci-Med.,Inc.

Man Years:

Total: 8
Professional: 3*
Other: 5*

*Does not include affiliated nursing and other professional care.

Project Description:

Objectives:

1. To evaluate the utility and liabilities, separately and together, of various procedures designed to reduce the incidence of severe infection in patients with acute leukemia receiving induction and/or consolidation chemotherapy; to evolve prophylactic techniques and equipment with greater utility than presently available. (6X1)(7XX)

2. To improve methods by which occult infectious agents may be detected and eradicated, e.g., latent herpes viruses, L-forms of bacteria, fastidious anaerobic bacteria, mycoplasma, and fungi. (6X1)(7XX)

3. To evaluate the human germ-free state, as it occurs in patients with leukemia, and to learn what is the influence of this state on normal physiologic and metabolic processes, and on the metabolism of cancer chemotherapeutic agents.

4. To improve our understanding of the natural history of unusual opportunistic infections, seen primarily in compromised hosts.

Methods Employed:

I. Protected Environment Study: Patients with early stages of leukemia, between 15 and 60 years of age, are randomly and concurrently assigned to receive induction chemotherapy under one of three conditions:

Group I: Patients receive antibacterial baths, sprays, and other modes of topical antisepsis and antibiosis, consume a diet low in bacteria, are confined within a laminar air flow isolator, and are given oral antibiotics (non-absorbable).

Group II: Patients are given the same oral non-absorbable antibiotic regime as patients in Group I, but are otherwise treated conventionally.

Group III: These patients are maintained in the usual ward setting, with no antimicrobial prophylaxis (control group).

Quantitative evaluation of the efficacy of prophylactic techniques is based upon comprehensive sampling of the patients and their environment for aerobic and anaerobic bacteria, fungi, mycoplasma, and viruses. Routine and special culture procedures are employed, as are serologic assays. Criteria for the diagnosis of infection are based on autopsy or biopsy results, cultures, x-rays, and clinical impression (in consultation with Dr. John Bennett, LCI, NIAID). Results are subjected to multiple statistical tests. In vivo and in vitro investigation of possible toxicity from prophylactic techniques is conducted.

II. Studies on Unusual Opportunistic Infections: Routine and special techniques are applied to the isolation of occult agents, immunofluorescent and other serologic assays, and animal innoculation. Such laboratory procedures are correlated with morphologic and cultural results gathered from pathologic specimens.

Major Findings:

I. Protected Environment Study

A. Efficacy of prophylactic components: The gentamycin, vancomycin, and nystatin regime continues to be extremely efficient in reducing the growth of aerobic and anaerobic bacteria in the stool. Virtually

100% of all such isolates are suppressed. Recently we have attempted to rinse the stool of antibiotic, and still find no growth of bacteria. However, it is likely that these organisms are not entirely eliminated from the gut since patients repopulate with serotypically identical organisms when the oral antibiotics are discontinued. The regime continues to be relatively ineffective in suppressing the growth of gut fungi although about 70-80% of stools cultured have not shown gut fungi. There has been no increase in the incidence of disseminated fungal infection in the antibiotic-treated patients. No untoward toxicity from the regime has been detected nor has the emergence of organisms resistant to the three antibiotics been noted. However, one bone marrow transplant recipient, housed in a LAF room (not a protocol patient) did demonstrate development of pseudomonas resistance to gentamycin. We continue to observe that the same stool culture results obtained, whether the patient is given a regular hospital diet or one low in bacteria, so long as the oral antibiotic regime is not a variable.

Regimes for skin antibiosis also continue to be highly effective,with more than 85% of skin samples remaining virtually sterile. However, results with orificial antisepsis are disappointing, with more than half the specimens (nasopharynx, etc.) containing significant numbers of aerobes, anaerobes, and fungi. Almost every patient studied has at least one body site in which organisms persist despite an intensive antibiotic and antiseptic regime. Since such sites are often the nidus for disseminated infection, much effort has been spent in identifying such sites and creating a unique eradication regime for each patient.

Four semi-portable laminar air flow rooms have been employed during the past year. Results to date confirm our previous experience with these units. Fewer than 4 organisms/1000 ft^3 are recovered from the air, as contrasted with 10,000/ft^3 in a conventional hospital room. Greater than 75% of all LAFR air samples are entirely sterile. More than 90% of floor, surface, and water samples are also sterile.

B. Results of the controlled study: 1. Number of induction trials completed: Group I, 27; II, 38; III, 30. Seventy-five percent of these trials were primary, following initial diagnosis, and 75% were in patients with AML (all 3 groups). The distribution of sex, age, and days spent at each granulocyte level were comparable for the 3 groups, as was the regime and total dosage of cancer chemotherapy.

2. Severe infection during induction chemotherapy (septicemia or organ invasion including extensive cellulitis and deep abscesses): There have been 10 episodes of severe infection in Group I or 0.43 episodes/patient, 30 in Group II or 0.81 episodes/patient, and 20 in Group III or 0.72/patient. Two episodes of sepsis occurred in Group I, as contrasted with 9 episodes of sepsis in Group II and 7 in

Group III. Two of the three pseudomonas infections in Group I occurred in patients in the "Life Island." The gram negative septicemia occurred in a patient who entered the LAFR with a heavy growth of Klebsiella in the pharynx and subsequently developed a Klebsiella glossitis followed by Klebsiella sepsis. In fact, there is no evidence that any Group I patient developed an infection caused by an organism acquired after he entered the barrier facility. Despite our inability to completely eliminate fungi with gastrointestinal antibiotics, no fungal infections were observed in Group I. No significant increase in the incidence of severe infection has been noted following removal from the protected environment or the withdrawal of gastrointestinal antibiotics.

We have recently reanalyzed the data, excluding patients who were treated in a "Life Island" and their concurrent controls, or who represented inadequate trials of the experimental situation. In this selected analysis, 17 patients in I had 0.41 total episodes of severe infection/patient, 25 patients in II had 0.88 episodes/patient, and 20 in III had 0.70 episodes/patient. There were 0.74 episodes/100 patient days in I, 2.09/100 patient days in II, and 1.69/100 patient days in III. When pneumonitis and/or sepsis was examined (the most frequent life-threatening infections), we found 0.12 episodes/patient in I, 0.52 in II, and 0.55 in III.

3. Infectious deaths: Of great importance was the finding that no documented infectious deaths occurred during induction chemotherapy of Group I patients out of 27 consecutive trials. Twenty-two percent of Group II and 27% of Group III patients died with severe infection as a primary cause of mortality. These diferences have a p value of <0.05. Many infectious deaths in III were caused by pseudomonas; in II, with the same low percentage of pseudomonas as in I, fatal infections were caused by a variety of organisms other than pseudomonas. These infections could usually be traced to a site other than the gut; It is probable that control patients (III) would have had a similar variety of infections had they not died of supervening pseudomonas infections. These observations demonstrate the efficacy of oral antibiotics, as the sole prophylactic technique, in reducing infection caused by enteric organisms; at the same time, they suggest that organisms in other sites will ultimately become apparent once the gut flora is reduced. It is in sterilizing these non-gut sites that the full regime offered to Group I patients has its greatest utility.

4. Days with severe infection and episodes of severe infection as functions of the granulocyte level: 45% of patient days were spent with severe infection in the control group (III), with granulocytes less than $100/mm^3$; also at this granulocyte level, there were 8 episodes of severe infection per 100 patient days on the study. These results indicate that the incidence of severe infection in leukemics at this institution is somewhat less than had been reported in earlier

studies from this and other institutions, thus emphasizing the need for concurrent, controlled studies. As anticipated, the incidence of severe infection was directly related to granulocyte level in all 3 groups. However, at granulocyte levels less than 100, only 1/4 as many days were spent with severe infection by Group I patients, as by controls, and only 1/4 as many episodes of infection were found in I. These data were statistically significant. Group II (oral antibiotic only), with granulocytes $<100/mm^3$, spent 60% of their days with severe infection. At higher granulocyte levels, similar, but less significant, trends were noted.

5. Results of induction chemotherapy: Despite the dramatic reduction in the incidence and lethality of infection in Group I patients, no significant inter-group differences were noted in the number of leukemic remissions obtained in this relatively small number of patients (95% of Group I patients survived for 100 days following entry in the study, as opposed to 65% in the other groups).

Conclusion: Highly encouraging results have been obtained with the use of a comprehensive prophylactic regime, including the use of laminar air flow isolation. These results have not been reflected in increased remission rates (it should be noted that protocol design required drug doses to be similar for all groups) but have augmented survival, such that subsequent induction attempts would become possible. Benefits have not been obtained from the use of oral antibiotics alone.

II. Transmission of Toxoplasmosis

Four patients with acute leukemia developed toxoplasmosis following leukocyte transfusions from donors with chronic myelogenous leukemia. Serologic data, obtained from the donors retrospectively, revealed elevated antitoxoplasma antibody titers, suggesting that the transfused leukocytes were the source of the organism in the recipients. Fatal recurrent toxoplasmosis in one leukemic patient apparently infected via a leukocyte transfusion from an asymptomatic carrier of the parasite was studied histologically. Although the initial illness resolved with supportive treatment and antimicrobial therapy, the recurrent phase was fulminating, resulting in colonization of many tissues. Acute necrotizing pancreatitis due to Toxoplasma gondii, not previously reported, was found. The biphasic nature of the infection was established on clinical as well as histologic grounds.

III. Longitudinal Cytomegalovirus Study

Cytomegalovirus (CMV) infections were studied longitudinally in 88 leukemic children. Over a mean observation time of 7.4 months, 24 patients had cultural evidence of CMV infection at some point during

study. There appeared to be a good correlation between virus excretion, either in urine and/or throat, and presence of complement-fixing (CF) and indirect-hemagglutination (IHA) antibody, although a small number of patients had detectable antibody without evidence of virus excretion or excreted virus but did not have detectable antibody. Compared to patients not excreting virus, patients shedding CMV had significantly more episodes of pneumonitis and fewer with rash, but not more episodes of hepatitis, fever of unknown origin, or upper respiratory tract infections. Clinical syndromes attributed to CMV correlated with four-fold rises in CMV antibody titer only during hematological remission. However, dissemination of CMV occurred during hematological relapse as well as during remission. Three patients who died with preterminal four-fold rises in CMV antibody had post-mortem evidence of dissemination. Virus excretion did not correlate with hematological relapse, appearance of clinical symptoms, antibody levels, four-fold rises in antibody or lymphocyte transformation against CMV. These data suggest that much information may be necessary to diagnose disseminated CMV infections occurring in acute childhood leukemia. Therefore, children with acute leukemia should be followed serially with both urine and throat cultures as well as repeated antibody determinations.

IV. Diagnosis of Systemic Candidiasis

A prospective study of anti-candida antibodies in patients with acute leukemia was completed. Correlations were made between cultural and pathological proof of invasive disease, and serological evidence of infection. The utility of the various serological procedures available was evaluated, and it was concluded that no present set of diagnostic criteria is reliably free of false positives or false negatives.

V. Mumps Virus Study

A 5-year-old boy who died with malignant lymphoma complicated by epidemic parotitis was the subject of this study. Mumps virus was isolated from the throat antemortem and from a parotid gland postmortem. Pathologic lesions were present in ductal and acinar cells of the parotid gland, submaxillary gland, and submucosal glands of the tracheobronchial tree. Ductal lining cells showed vacuolization, degeneration, and focal desquamation. Acinar cells exhibited severe cytoplasmic vacuolization. Other changes included sialectasia, duct obstruction, and periductal edema. Electron microscopic studies revealed disruption of cellular organelles and large cytoplasmic vacuoles which appeared to arise from dilated endoplasmic reticulum. Particles consistent with myxoviruses were present in ductal and acinar lumens. The evidence suggests that the predominate lesion of mumps parotitis is vacuolar degeneration of acinar and ductal epithelium. Such observations have not hitherto been made, since death with or due to mumps virus, is a rare event.

Proposed Course:

1. The controlled, randomized study of protected environments has now reached statistical significance, in all 3 groups, for most parameters under study. The data strongly suggest the utility of a comprehensive antimicrobial regime, including laminar air-flow isolation, in reducing mortality and morbidity due to infection. At the same time, oral antibiotics alone appear to offer no benefit to the patient and in fact may be deleterious. Consequently, we have completed the initial study and will discontinue the use of oral antibiotics alone.

   In the recently concluded study, chemotherapy was deferred because of granulocytopenia rather than infection per se. Consequently, all 3 groups of the study received approximately the same total dose of any one agent. It is possible that the reduction in severe infectious episodes noted in Group I will eventuate in an improved antileukemic result (higher remission rate and longer remission duration), if a greater total dose of therapy can be achieved prior to infection than is possible in the unprotected patient. However, the Group I regimen can significantly benefit those patients whose only reason for failing to remit is supervening infection. The ultimate benefit of this program therefore, awaits improvements in chemotherapy per se.

   The study to be instituted in 1972 is designed to provide continuing information on the utility of the Group I regimen, as antileukemic therapy evolves. Accordingly, we shall administer chemotherapy to Group I with greater intensity than in Groups II or III. Groups II and III will not differ regarding chemotherapy and will thus provide continuing information on rates of infection without chemotherapy as a variable. Finally, studies will be directed toward more thorough bacterial, viral, and fungal decontamination of patients. Most infections which did occur in a protected environment during the 1968-71 study could be traced to an obvious nidus which the patient had upon entry to the isolation room. There was no evidence that any patient became contaminated once in the room.

   Design of Study: Eligible patients will be randomly assigned to receive induction chemotherapy under the following scheme:

Group I	Laminar Air Flow Room (LFR) plus topical and oral prophylactic antibiotics, plus low bacterial diets; superintensive[1/] induction chemotherapy (Ara-C and 6-TG).

   1/ Instead of stopping therapy when marrow is aplastic, therapy continues q 12 h for a number of additional days equal to the time it took for the patient to reach this bone marrow status (from the onset of therapy). The maximum additional time of therapy beyond the point of hypoplasia will be limited to 7 days. As with II and III, therapy will be stopped when severe infection supervenes.

Group II — As above; conventional[2/] Ara-C and 6-TG (LS-72-4).

Group III — Ward care with no prophylactic antibiotics (control); conventional[2/] Ara-C and 6-TG induction (LS-72-4).

2. As facilities become available, we hope to include a small number of patients in protected environment studies who are not candidates for the randomized induction study. Such patients will include bone marrow transplant recipients and patients with other malignant diseases who are at risk of infection.

3. Other areas of investigation in 1972-73 will include:

   a) New antibiotic trials, e.g., Nebramycin Factor 6;
   b) Limulus assay for endotoxin;
   c) Photoinactivation of herpes simplex (recurrent);
   d) Clinical trials of zoster immune-globulin and leukocyte interferon;
   e) Metabolism and transport of anti-pseudomonas immunoglobulins.

4. As contract facilities become available, we plan to undertake gnotobiological studies in small animals, with a view to manipulative proceedures that might render deleterious organisms harmless in terms of patient morbidity, but permit more normal physiology than the germ-free state. Suth procedures might include ablation and recolonization, and the use of selective overgrowth of innocuous organism. We also plan to investigate, in collaboration with contract facilities, the alterations in metabolism, immune competence and chemotherapeutic toxicity which may result from the germ-free state.

5. Certain infectious problems encountered clinically may be approached diagnostically and/or therapeutically, using the tools of molecular biology. We intend to use such tools in exploring the latent state of the human herpes virus, in patients, and in attempting a screen of banked blood products for latent herpes genetic material (e.g., CMV) that may be transmissible.

   Another area of interest will be the genetic manipulation of noxious bacteria by phage infection.

2/ 100 mg/M^2 Ara-C by rapid IV injection plus 90 mg/M^2 6-TG p.o. (60 mg/M^2 IV); both drugs given simultaneously every 12 hours until the bone marrow is severely hypoplastic, i.e., contains no normal or abnormal hematopoietic precursor cells, unless severe infection occurs (documented sepsis or organ invasion). Repeat courses to be administered as above, allowing for recovery from marrow toxicity between courses, until remission is achieved or there is definite evidence of disease progression.

Honors and Awards:

Invited Lectures:

1. Laminar Air-Flow Rooms and Gastrointestinal Antibiosis in the Therapy of Acute Leukemia. Presented at the 13th International Congress of Pediatrics, Vienna, September 1971 (A. S. Levine).

2. Supportive Care of Leukemic Patients. Presented at the Raleigh Academy of Medicine, November 1971 (E. S. Henderson).

3. Protected Environments. Presented in Melbourne, Australia, 1971 (E. S. Henderson).

4. Infections in Patients with Leukemia and Lymphoma. Seminar at Johns Hopkins Medical School, Division of Hematology, November 1971 (S. Siegel).

5. Infections in Patients with Leukemia and Lymphoma. U.S.C. School of Medicine, Department of Pediatrics, December 1971 (S. Siegel).

6. Report on Laminar Air Flow Studies. Presented before the Advisory Cancer Council, NIH, October 1971, (A. S. Levine).

7. Infectious Complications in the Altered Host-Experimental Approaches. George Washington University School of Medicine, Department of Pharmacology Series, April 24, 1971 (A. S. Levine).

Publications:

1. Siegel, S., Lunde, M., Gelderman, A., Halterman, R., Brown, J., Levine, A., and Graw, R.: Transmission of toxoplasmosis by leukocyte transfusions. Blood 37: 388-394, 1971.

2. Roth, J., Siegel, S., Levine, A., and Berard, C.: Fatal recurrent toxoplasmosis in a patient initially infected via a leukocyte transfusion. Am. J. Clin. Path. 56: 601-605, 1971.

3. Preisler, H. D., Bruckner, H., and Henderson, E. S.: Reduced production of spleen colonies and granulocytes in antibiotic-treated irradiated mice transplanted with syngeneic bone marrow. Radiation Res. 48: 20-31, 1971.

4. Preisler, H. D., Hasenclever, H., and Henderson, E. S.: Anticandida antibodies in patients with acute leukemia: A prospective study. Am. J. Med. 51: 352-361, 1971.

5. Levine, A. S., Schreiber, A., Goldstein, I., Preisler, H., and Henderson, E. S.: Laminar air-flow rooms and gastrointestinal antibiosis in the therapy of acute leukemia. Proc. 13th Int. Congr. Ped. 14: 165-169, 1971.

6. Levine, A. S., Graw, R. G., Jr., and Young, R. C.: Management of infections in patients with leukemia and lymphoma: Current concepts and experimental approaches. Seminars in Hemat. (in press).

7. Henson, D., Siegel, S., Fucillo, D., Mathew, E., and Levine, A. S.: Cytomegalovirus infections during acute childhood leukemia. J. Infect. Dis. (in press).

Serial No. NCI-3426
1. Hematology & Supportive Care Branch
2. Clinical Oncology, Chemotherapy, NCI
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Hemorrhagic Complications of Acute Leukemia

Previous Serial Number: Same

Principal Investigators: Ronald A. Yankee, M.D., and Edward S. Henderson,M.D.

Other Investigators: Kenneth Graff, M.D., Roger Halterman, M.D., Brigid Leventhal, M.D., and Harvey Gralnick, M.D.

Cooperating Units: Blood Bank, CC
American National Red Cross, Washington, D.C.
Central Blood Service, Baltimore, Maryland
DCRT (NIH)
Immunology Branch, NCI
Microbiological Laboratories, Bethesda, Maryland
University of Los Angeles, Los Angeles, California

Man Years:

Total: 4
Professional: 2 1/2
Others: 1 1/2

Project Description:

Objectives:

To improve methods of preventing or controlling thrombocytopenic hemorrhage in patients with leukemia and other malignancies. Especially to determine methods for the selection of compatible donors from the general population. (6X1)

Major Findings:

I. Long Term Platelet Support: The efficacy of HL-A matched sibling platelets in the support of patients refractory to random donor platelets has been further documented. One patient with aplastic anemia who received 4 units of platelets from each of two matched sibs for 4 years has had a complete bone marrow and peripheral blood recovery and is presumed cured of her disease. A second patient transfused weekly

with 8 units of matched sib platelets from one donor for 3 1/2 years has had a partial normalization of erythroid and myeloid elements but continues to require platelet support. These two cases establish the feasibility of long term platelet maintenance in patients who have the potential for bone marrow recovery.

II. Selection of Compatible Platelet Donors by HL-A Typing: The hypothesis that HL-A lymphocyte typing can be used as a means of selecting compatible platelet donors from unrelated individuals has not as yet been clinically documented. In the past two years 2,536 blood bank donors were HL-A typed. Three thrombocytopenic patients refractory to platelet transfusions from unmatched random donors received matched platelets from those HL-A typed individuals who had at least 2 antigens defined with no antigens different from those of the recipients. The response to repetitive transfusions was measured for a period of 60 to 90 days. The median response (Inc x m^2/u) to matched donor platelets was 14,000/mm^3 where as the response to non matched donor platelets given during the same period was < 1,000/mm^3. These studies provide the basic documentation that the HL-A system is clinically the most important system of antigens in determing platelet compatibility. Furthermore, for the first time, a practical system of typing has been successfully developed in selecting compatible platelets for refractory patients from a panel of non family donors.

III. Alloimmunization to Platelet Transfusions: The pattern of alloimmunization to HL-A antigens was studied in a previously non transfused patient with aplastic anemia. The patient received platelets from only one unrelated non matched donor twice weekly for 9 weeks. A leukoagglutinin against the donor developed within the first week, lymphocytotoxic antibody by the second week and an increase in MLC reactivity by the third week. The patient continued to respond to the donor platelets despite high levels of both granulocyte and lymphocyte antibody directed specifically against the donor. This observation suggests that the development of HL-A antibody is cell specific i.e. the presence of a specific lymphocyte antibody may not reflect the presence of platelet antibody of the same specificity. Thus, although lymphocyte typing appears to essential for long term compatibility, the presence or absence of antibody against non matched donor lymphocytes is not useful in predicting platelet compatibility or incompatibility from that donor.

IV. Selection of Compatible Platelets from a Population of Donors Enrolled in a Repetitive Plateletpheresis Program: This program represents the major mechanism for providing more compatible platelets for routine platelet therapy. Typed donor platelets received daily can be selected for each recipient. Limited control over antigen exposure can thus be achieved and alloimmunization against specific antigen types can thus be tested. Serial sera from all patients transfused is tested weekly for the presence of lymphocytotoxic and platelet complement fixing antibodies. Knowledge of the presence of antibody is utilized in selecting donors to avoid transfusion of incompatible platelets.

V. HL-A Typing and Disease: The HL-A antigens were studied in families of patients with leukemia and Hodgkins disease. Patients with acute lymphocytic leukemia have an increased frequency of HL-A 2. Patients with Hodgkins disease have an increased frequency of HL-A 5. However, HL-A 5 is significantly decreased in patients with nodular sclerosis compared to lymphocyte predominance and mixed cellularity. These observations suggest as found in animals, a relationship between disease and the HL-A system. The significance of these findings is not clear.

VI. Isolation of Fab Fractions and Tolerance Induction Fab Fractions of Patient Serum: Fab fractions of patient serum containing specific antibody has been used to coat platelts in an attempt to induce tolerance to individual donor platelets. This method has not been clinically successful on two occassions. Platelet transfusions scheduled with chemotherapy to induce tolerance to platelet antigens has been initiated. These results cannot be evaluated at the present time.

VII. Evaluation of the Latham Machine Plateletpheresis: Ten donors were studied each by the 4 unit plateletpheresis procedure and again by the Latham machine plateletpheresis procedure. These studies have just been completed and have not been fully evaluated. Preliminary results suggest little or no advantage to the machine procedure in the yield of platelets per unit time.

Proposed Course of the Project:

1) Determine a practical approach to platelet therapy that will incorporate knowledge of HL-A alloimmunization and the mechanisms for selecting compatible platelet donors.

2) Confirm and establish further the relative effectiveness of matched random donor platelets.

3) Continue evaluation of HL-A antigen frequency and disease.

4) Explore the induction of enhancing antibody and the development of cytotoxic antibody as it relates to transfusion therapy with particular emphasis on its utilization in providing more effective platelet support.

Publications:

1) Yankee, R.A.: The importance of histocompatibility in platelet transfusion therapy. Vox. Sang. 20: 419, 1971.

2) Skeel, R.T., Yankee, R.A., and Henderson, E.S.: Hexosemonophosphate shunt activity of circulating phagocytes in acute leukemia. J. Lab. Clin. Med. 77: 975-984, 1971.

3) Halterman, R.H., Grumet, F.C., Watson, L., Yanchulis, F., and Yankee, R.A.: Comparison of methods for the preparation of leukocyte poor blood. Transfusion 12: 23, 1971.

4) Henderson, E.S., and Goldstein, I.: Platelets in leukemia. In The Platelet, Brinkhaus, K.M. and Mostofi, F.K., Williams & Wilkins, Baltimore, 1971, pp. 315-343.

5) Wunderlich, J.R., Rogentine, G.N., Jr., and Yankee, R.A.: Rapid in vitro detection of cellular immunity to man against freshly explanted allogeneic cells. Transplantation 13: 31-37, 1972.

6) Rogentine, G.N., Jr., Yankee, R.A., Gart, J.J., Nam, J., and Trapani, R.J.: HL-A antigens and disease. Acute lymphocytic leukemia. (Accepted for publication J. Clin. Invest).

Serial No. NCI-6301
1. Hematology & Supportive Care Branch
2. Clinical Oncology, Chemotherapy, NCI
3. Bethesda, Maryland

PHS-NCI
Individual Project Support
July 1, 1971 through June 30, 1972

Project Title: Transplantation Biology

Previous Serial Number: NCI-3419 and NCI-6300

Principal Investigators: Robert G. Graw, Jr., M.D., Geoffrey P. Herzig, M.D. Robert L. Carolla, M.D., Edward S. Henderson, M.D., Thor A. Alvegard, M.D., Brigid G. Leventhal, M.D., Ronald A. Yankee, M.D., Roger H. Halterman, M.D., and Joan M. Bull, M.D.

Other Investigators: G. Nicholas Rogentine, M.D., Christopher Merritt, M.D., Gerhard Krueger, M.D., Costan Berard, M.D., Harvey Gralnick, M.D., Jacqueline Whang-Peng, M.D., Mary McGinniss, Regina Dowling, Ruth Estabrook, Carole Novick, Shirley House, Pamela Woodruff, Thomas Pomeroy, M.D., David Johnson, D.V.M., Scott Volker, Larry Brinkman, Frank Stout, P.J. Scullen, Richard Brooks, Eugene Rush, James Reid, M.D., John Nutter, Harry Hughes, Everett Johnson, Paul I. Terasaki, M.D., Arthur Steinberg, Jack Smith,M.D. Cecilia Lester, and Walter Stark, M.D.

Cooperating Units: Medicine Branch, NCI
Immunology Branch, NCI
Laboratory of Pathology, NCI
Hematology Service, Clinical Pathology, CC, NCI
Blood Bank, CC, NIH
Anesthesiology Service, NIH
National Institute of Allergy and Infectious Diseases
Department of Surgery, University of California at Los Angeles
Department of Medicine and Surgery, Division of Research Services, NIH
Plasmapheresis Unit, NCI
Dermatology Branch, NCI
Radiation Branch, NCI
Western Reserve School of Medicine, Cleveland, Ohio
Armed Forces Radiobiology Institute; Tissue Bank and Naval Research Institute, National Naval Medical Center; The Armed Forces Institute of Pathology
Microbiological Associates, Bethesda

Bionetics Research Laboratories, Rockville
Hazelton Laboratories, Falls Church, Virginia
National Eye Institute, NIH

Man Years:

Total: 10
Professional: 3
Other: 7

Project Description:

Objectives:

1) To assess the optimal methods of obtaining and maintaining allogeneic and isogeneic bone marrow grafts in humans.

2) To assess the use of marrow stem cell grafting as an adjunctive measure in the treatment of leukemia and other disorders of hematopoiesis.

3) To examine the role of HL-A matching for the selection of compatible bone marrow donors.

4) To study the complex immunologic reaction of grafted tissue known as graft-versus-host reaction (GVH) as influenced by histocompatibility matching of donors and recipients for transplantation antigens; transfusion of supportive blood products; use of immunosuppressive agents post transplant;and other modifications of the host's immune defenses.

5) To examine the usefulness of frozen autologous bone marrow in the management of patients with malignancy.

6) To examine a random breed large animal model (canine) as a tool to study factors influencing bone marrow graft acceptance, rejection and the development of GVH as the findings may apply to humans.

7) To study the anti-tumor capability of allogeneic bone marrow transplantation in spontaneous neoplasms occurring in the canine model system.

8) To delineate the influence of blood component transfusions on the effectiveness and complications of bone marrow transplantation.

Methods Employed

1) Clinical trials of allogeneic bone marrow transplantation from siblings matched for histocompatibility antigens (HL-A and MLC studies) to patients with leukemia refractory to conventional chemotherapy.

2) Autologous bone marrow storage in liquid nitrogen to be utilized as an adjunct to chemotherapy protocols.

3) Preclinical evaluation of frozen autologous bone marrow in canine and primate models following cyclophosphamide and/or total body gamma irradiation.

4) Preclinical allogeneic bone marrow transplantation program continued using the large animal model system (canine) to examine various forms of immunosuppression to be used for pre-transplant immunosuppression and post-transplant management of graft-versus-host disease; to study the incidence and spectrum of GVH disease using histoincompatible and histocompatible (DL-A) donor-recipient pairs; to study the process of in vivo sensitization to transfused blood products and through transplantation of skin and corneas; and to establish models to study the possibility of "marrow-rescue" techniques applicable for human trials.

Major Findings:

1) Two additional HL-A and MLC compatible allogeneic bone marrow transplants have been performed in multiply transfused patients with leukemia in an effort to evaluate a possible antileukemic effect.

The first patient, an 18 year old boy with acute myelogenous leukemia (AML) was transplanted from his 16 year old male sibling, matched for histocompatibility antigens. The primary immunosuppressive protocol was altered from the four-day cytoxan regimen used in previous patients since a previous patient with acute myelogenous leukemia relapsed soon after completion of the regimen and since other patients successfully engrafted with cyclophosphamide alone demonstrated states of "mixed" chimerism. The new drug protocol incorporated the usual four day course of cyclophosphamide (45 mg/kg/day) combined with twice daily doses of cytosine arabinoside (100 mg/m^2) and 6 thioguanine (100 mg/m^2) and a single dose of BCNU (200 mg/m^2) on the last day of the regimen.

The patient experienced severe but reversible gastrointestinal toxicity and was successfully engrafted. During the first post-transplant month he developed severe graft-versus-host disease which was successfully managed with the use of anti-lymphocyte serum, plus passively administered naturally occurring "enhancing-blocking" antibodies which has been collected from his mother prior to transplantation. This is the first NCI transplant candidate placed in the laminar air flow room for patient isolation during the immunosuppressive period and was also given intravenous hyperalimentation during the period of graft-versus-host disease. At this time, he is 215+ days post transplant in clinical remission from disease (this patient represents the longest AML remission following HL-A matched bone marrow transplantation). In an attempt to document levels of host reactivity against his primary tumor during the post-engraftment period the reconstituted patient (chimera) was studied for

MLC responsiveness (peripheral blood lymphocytes against primary tumor cells stored in liquid nitrogen before transplantation). The level of reactivity were similar to that seen in patients followed for autologous reactivity against tumor, and suggest reconstitution of antitumor immunologic reactivity through marrow grafting. A second assay of reactivity of graft-versus-host disease consisted of serial measurement of two hour spontaneous DNA synthesis of peripheral blood lymphocytes throughout the transplant period. Preliminary results indicate that this assay may have usefulness in predicting exacerbations of GVH and in evaluating various forms of immunosuppressive therapy for the management of GVH. During the post transplant period he has demonstrated a moderate degree of immunologic incompetence manifested through decreased levels of secretory IgA and a moderate deficiency of granulocyte function characterized by a low level of granulocyte phagocytosis and bactericidal capacity. A majority of his post-transplant period has been spent outside of the hospital in his home in North Carolina.

The second patient transplanted with HL-A identical marrow is a patient on the Medicine Branch of the Clinical Oncology area who has been treated for the blastic phase of chronic myelogenous leukemia for the six months prior to transplantation. During the initial chemotherapy of his blastic leukemic phase he received cytosine arabinoside and 6 thioguanine (6 TG) which were used to maximum marrow and extramedullary toxicity producing severe pancytopenia requiring blood component transfusions. Because of prior sensitization to random donor blood component products he was supported during the pre-transplant period with platelets and leukocytes collected from two HL-A identical brothers. A random donor, phenotypically matched for HL-A antigens, was also successfully used to manage states of thrombocytopenia but could not be used for granulocyte support (most probably because of sensitization to granulocyte specific antigens). After recovery of moderately normal bone marrow function and a three week rehabilitation period the patient was prepared for bone marrow transplantation using 1000 rads of gamma irradiation from a van de Graf generator. Radiotherapy was given without complication, and HL-A and MLC compatible bone marrow from a third HL-A identical sister was infused. At the time of this report the patient is twelve days post transplant with evidence of tumor eradication most probably resulting from the gamma irradiation. He has developed no sequelae of graft-versus-host disease or complications attributable to extra medullary toxicity.

Both patients were treated with post-transplant immunosuppressive drug regimens the first with cyclophosphamide and the second with a modified methotrexate regimen. Since clinical evidence to date has not documented whether these chemotherapy regimens are required for the management of graft-versus-host disease a randomized protocol has been written to study the efficacy of each.

2) Autologous bone marrows continue to be harvested and stored in liquid nitrogen on patients with acute leukemia. This bone marrow is to be

used as a safety measure for those patients undergoing HL-A matched sibling bone marrow transplantation. In the event a patient develops life threatening GVH it is planned to destroy the graft causing severe graft-versus-host disease and repopulate the patient with his stored autologous bone marrow, fully recognizing that this autologous marrow may have minimal number of leukemic cells with proliferative capacity. Patients not having phenotypically HL-A matched or otherwise suitable bone marrow donors (approximately 85% of NCI families) are having autologous bone marrow stored during periods of clinical remission from leukemia in order that the antileukemic effect of the primary immunosuppressive regimens used for transplantation can be evaluated using a form of marrow repopulation which should offer no immunotherapeutic advantage. As of this date thre have been no human autologous marrow infusions given.

3) Bone marrow frozen on canine and primate donors has been successfully used on numerous occasions to repopulate animals given lethal doses of cyclophosphamide or total body X-irradiation (800 rads). Frozen primate bone marrow has been successfully administered to monkeys following a single dose of cyclophosphamide (45 mg/kg) given four days after allogeneic bone marrow transplantation. A significant number of these animals (approximately 75%) were able to successfully repopulate their marrows with hematopoietic tissue of original host origin. Similar studies in the canine model system have attempted to eradicate viable bone marrow graft which have been documented by progressively rising peripheral white blood cell and reticulocyte counts, bone marrow karyotypes and donor type red blood cell markers. In the canine model system, with documented evidence of engraftment and clinical manifestations of graft-versus-host reaction at the time marrow eradication is attempted, it appears that it is critical to provide continuous granulocyte and platelet transfusion support for the periods of pancytopenia. The best regimen found requires that support be given simultaneous with three consecutive days of cyclophosphamide (15 mg/kg/day) used to destroy the graft before giving frozen autologous bone marrow. It is anticipated that this animal model system can be applied to animals bearing spontaneous neoplasms to study the immunoadjuvant effect of transplanted allogeneic bone marrow and controlled graft-versus-host disease.

4) The pre-clinical canine model has been extensively used to evaluate other factors contributing to successful marrow engraftment as follows:

A. Primary Immunosuppression for Marrow Transplantation

Two forms of primary immunosuppression have been examined in depth, namely lethal doses of cyclophosphamide and total body gamma irradiation. It appears from the initial studies that levels of cyclophosphamide required for adequate and complete immunosuppression of the host are associated with severe extramedullary toxicity limiting its usefulness as a safe and reproducible single immunosuppressant. 700 to 1200 rads (midline tissue dose) of total body Cobalt 60 gamma

radiation were examined to determine the level which would produce adequate immunosuppression with the least extramedullary toxicity. Animals receiving doses in excess of 800 rads (46 R/min) from bilaterial opposing Cobalt 60 sources experienced a high incidence of pulmonary and gastrointestinal toxicity. This toxicity was augmented when post transplant immunosuppressive agents were added in an attempt to modify anticipated graft-versus-host disease. Animals receiving the safe and reproducibly effective dose of 800 rads have experienced a high incidence of spontaneous graft rejection, 10 to 20 days post transplant. Additional studies have demonstrated that the addition of L-asparaginase post transplant (2000-5000 units/dog/day X 10 days post transplant, intravenously) provided reproducible and effective marrow engraftments. No dogs receiving the regimen of 800 rads TBI plus 10 days of L-asparaginase and adequate marrow dose have rejected transplanted histoincompatible or histocompatible marrow. Dogs receiving doses of bone marrow less than that found to be optimally required for allogeneic transplantation (> 7 X 10^9 cells/dog) have been successfully engrafted using the X-rays + L-asparaginase combination. Laboratory investigations in progress will attempt to define the minimum dose of L-asparaginase which can be utilized to provide the additional immunosuppression required to produce effective marrow transplantation. As used in the described fashion, L-asparaginase has not demonstrated any effect against graft-versus-host disease occurring with histoincompatible grafts.

B. Antilymphocyte Serum

Anti dog thymocyte serum has been raised in 4 animal species (burro, goat, rabbit and horse) in order to determine which preparation would offer consistent and reproducible immunosuppression for transplantation. Each batch of serum (most animals boosted with 10^9 thymocytes at monthly intervals following the initial immunization schedule) have been assayed in vitro for lymphocytotoxicity; and rosette the inhibition activity an in vivo by prolongation of skin allografts. The best sera prepared to date have come from horses (skin grafts prolongation (SGP)= 20-30 days) and rabbits (SGP = 55-60+days) as compared to controls with a median of 9 days. No marrow transplants have been performed to date using these ALS preparations.

C. Other Types of Immunosuppression

1) Naturally occurring "blocking" antibodies have been discovered in the sera of canine mothers, which will block mixed leukocyte culture reactivity against offspring. Two marrow transplants were done in an attempt to use this antibody which could be plasmapheresed from the mothers in large volumes. The first was a transplant from the mother to an offspring, and the second was an attempt to use the antibody to block GVH occurring following transplantation of marrow between mismatched littermates. In

both cases, one unit of plasma was transfused intravenously daily post transplant, but failed to interfere with the progression of GVH and the animals died.

2) "Anti light chain antibodies" have been shown to be effective in murine systems in blocking GVH by in vitro incubation of antibody and bone marrow before infusion. Anti dog immunoglobulin sera (light chain, gamma blobulin, and IgG) have been prepared in sheep and shown to have specificity against the immunizing preparations in Octerlony plates, with no lymphocytotoxicity toward canine cells. However, the preparations raised appear to be toxic to dog bone marrow cells grown in vitro. Using a non-toxic concentration of anti light chain antibody, we have been unable to prevent GVH disease in a single animal transplanted from a mismatched donor.

D. Post Transplant Immunosuppression

1) Methotrexate has been extensively tested for its ability to enhance marrow engraftment and modify GVH. In all dose ranges and schedules tested (0.2 mg/kg - 0.5 mg/kg and 1 day versus day 1, 3, 6, versus day 1, 3, 6 and weekly) all animals tested (35) demonstrated severe and lethal gastrointestinal toxicity to this agent when added to 800 rads TBI for allografting. These findings have raised the question of extra medullary toxicity resulting from TBI determined possibly by the rate of x-ray administration (9-46 rads/min), an observation previously unrecognized until MTX was added post-transplant. Studies of TBI using different total doses and rates (800 rad - 46 R/min versus 900-1000 rad-9R/min) have shown that methotrexate in doses recommended for clinical use becomes extremely toxic when added to doses of x-ray which provide sufficient immunosuppression, regardless of which dose rate is used.

E. Post-transplant Supportive Care

1) A humeral factor in the circulation of animals on day 4-7 post total body irradiation has been found which stimulates in vitro marrow colony growth. This factor harvested in large quantities from animals given TBI was used in an attempt to accelerate marrow repopulation in animals given marrow transplants. In four animals receiving autografts post TBI transfused with 250 ml of plasma containing this factor on day 0, 1, 2 and 3 post transplant, there was no evidence of toxic effect on marrow growth and in each case marrow repopulation occurred very rapidly (5-6 days). Whether similar substances can be collected from patients receiving chemotherapy or following extensive leukapheresis for future autotransfusion during periods of marrow hypoplasia remains to be examined.

2) The influence of supportive transfusions and prophylactic antibiotics have been examined in beagles irradiated with 350 rads and 500 rads. All animals not given antibiotics or transfusion support have died in each group (6/6). Animals given 350 rads could be saved from an apparent hematopoietic death (8/9 survivors) when given irradiated RBC, and platelet transfusions in conjunction with parenteral ampicillin (beginning day 6) and gentamicin (beginning day 12) post TBI. In no case could animals given 500 rad be carried through post TBI toxicity with the same measures (15/15 deaths). Preliminary efforts to support animals in the second group with canine granulocyte transfusions have been complicated by the development of anti-leukocyte antibodies (3-4 days following the first transfusion, which was given 13 days post TBI). This model for an accelerated development of antibodies to blood components closely approximates that seen in patients given transfusions post chemotherapy and will be used to study mechanisms of antibody induction and methods to prevent or modify sensitization to transfusion.

F. Assays of Histocompatibility

1) In an attempt to recognize and detect antigenic difference existing between supposed matched (DL-A or HL-A) donor-recipient pairs. We have examined the mixed leukocyte-buccal mucosal cell culture for its ability to detect minor antigenic differences unrecognized by the mixed lymphocyte culture alone. Using trypsinized and ultrasonically treated buccal cell preparations we have been unable to recognize histocompatibility differences.

2) A combination of assays (MLC and in vitro lymphocyte cellular cytotoxicity) has been successfully used to detect subtle antigenic differences existing between DL-A matched (MLC non-stimulatory) non-littermates. Whether this new assay can be used to determine the levels of histoincompatibility existing between DL-A matched littermates and HL-A matched siblings which can cause fatal GVH is under investigation.

As presented above these experimental animal models provide an opportunity to manipulate and examine in detail pre-transplant environmental conditions, supportive factors thought to influence survival and other important variables which must be better understood before marrow allografting can be generally applied to patients with disordered hematopoesis. The ability to rapidly answer questions in an animal model offers greater safety for patients being managed with these experimental procedures.

Proposed Course

1) Continue HL-A and MLC compatible bone marrow transplants in patients with

malignancy or marrow failure refractory to standard therapy.

2) Continue investigations with autologous bone marrow grafting in patients with malignancy where no HL-A compatible donor can be found or in an attempt to modify severe forms of GVH disease.

3) Continue preclinical evaluation of allogeneic bone marrow grafting, particularly focusing on factors influencing marrow acceptance, graft-versus-host disease and its relation to antitumor reactivity. Continuing studies include:

   a. Evaluation of the influence of DL-A matching on the graft-versus-host reaction and management of animals through post-transplant complications.

   b. Continued evaluation of various forms of pre and post transplant immunosuppression with particular emphasis on methotrexate, L-asparaginase, cyclophosphamide, procarbazine and antilymphocyte serum.

   c. Initiation of marrow grafting experiments using antilymphocyte serum preparations alone or in combination with other forms of cytotoxic immunosuppressive agents. Increased effort at categorizing the immunosuppressive potency of the various antilymphocyte serum preparations prepared during the previous year for applicability to transplantation.

   d. Continued examination of "marrow rescue" techniques for the management of graft-versus-host disease with an application to animals bearing spontaneous neoplasms.

   e. An evaluation of the use of enhancing antibodies in transplant and transfusion recipients.

   f. Begin evaluation of "in vitro" suicide experiments.

   g. Continued evaluation of in vitro assays which may define histoincompatibility responsible for graft-versus-host reaction existing between apparent DL-A and MLC matched canine pairs and HL-A and MLC matched human transplant recipient pairs.

   h. Attempt to devise new assays and continue an analysis of current assays for determination of histocompatibility and sensitization to histocompatibility antigens which may hinder engraftment or transfusions of histocompatible or histoincompatible hematopoietic tissue.

   i. Continued evaluation of immune competence of chimeric patients following bone marrow transplantation. Further attempts will be made to delineate the reactivity of marrow allograft recipients against transplanted tissue and reactivity of donor cells against the

host in order to follow the levels of "graft rejection" or "graft-versus-host" reactivity.

j. Initiate an investigation of human and canine soluble transplantation antigens in order to study their role in determining a donor-recipient "tolerant" state.

Honors and Awards:

Invited Lectures
Robert G. Graw, Jr., M.D.

1. American Society of Hematology, Symposium on Bone Marrow Transplantation. Bone Marrow Transplantation in Patients with Acute Leukemia Employing Cyclophosphamide. San Francisco, California, December 4, 1971.

2. Australian-U.S. Scientific Information Exchange. Seminar on Biochemical and Immunological Advances in Cancer. Bone Marrow Transplantation for the Treatment of Malignancy. Melbourne, Australia, March 22, 1972.

Geoffrey P. Herzig, M.D.

1. American Society of Hematology, Use of Frozen Autologous Bone Marrow to Rescue Monkeys from Acute Graft versus Host Disease. San Francisco, California, December 5, 1971. Proceedings of ASH-#77.

2. American Federation Clinical Research, Allogeneic Bone Marrow Transplantation (BMTX) Following Total Body Irradiation (TBI) and L-Asparaginase (L-ASP). Clinical Research 20: 490, 1972, Atlantic City, New Jersey, May 1972.

Publications:

1) Graw, R.G., Jr., Leventhal, B.G., Yankee, R.A., Rogentine, G.N., Whang-Peng, J., McGinniss, M.H., Herzig, G.P., Halterman, R.H., and Henderson, E.S.: HL-A and mixed leukocyte culture matched allogeneic bone marrow transplantation in patients with acute leukemia. Transplantation Proc. 3: 405-408, 1971.

2) Krueger, G.R.F., Berard, C.W., Elias, P.M.,Graw, R.G., Jr., Rogentine, G.N., Leventhal, B.G., Yankee, R.A., Herzig, G.P., Halterman, R.H., and Henderson, E.S.: Morphology of graft-versus host reaction in HL-A matched bone marrow transplantation and its differential diagnosis. Exp. Hemat. 21: 4-7, 1971.

3) Graw, R.G., Jr., Yankee, R.A., Leventhal, B.G., Rogentine, G.N., Herzig, G.P., Halterman, R.H., Krueger, G., Berard, C.W., and

Henderson, E.S.: Graft-versus-host disease complicating HL-A matched bone marrow transplantation. Exp. Hemat. 21: 11-13, 1971.

4) Halterman, R.H., Leventhal, B.G., Graw, R.G., Jr., Yankee, R.A., Rogentine, G.N., Herzig, G.P., and Henderson, E.S.: Immunocompetence of patients following HL-A matched bone marrow transplantation. Exp. Hemat. 21: 13-14, 1971.

5) Rogentine, G.N., Merritt, C.B., Rosenberg, S.A., Yankee, R.A., Leventhal, B.G., Graw, R.G., Griepp, P., Whang-Peng, J., and Fahey, J.L.: Allogeneic bone marrow transplantation in aplastic anemia. A case report. Exp. Hemat. 21: 21, 1971.

6) Graw, R.G., Jr., Yankee, R.A., Leventhal, B.G., Rogentine, G.N., Herzig, G.P., Halterman, R.H., Merritt, C.B., Carolla, R.L., Alvegård, T.A., Bull, J.M., McGinniss, M.H., Krueger, G.R.D., Guillion, D.S., Lippman, M.E., Berard, C.W., Whang-Peng, J., Trapani, R.J., Terasaki, P.I., Steinberg, A.S., Gralnick, H.R., and Henderson, E.S.: Bone marrow transplantation in acute leukemia employing cyclophosphamide. Exp. Hemat. 22: 118-125, 1972.

7) Merritt, C.B., Herzig, G.P., Darrow, C.C., and Rogentine, G.N., Jr.: Use of frozen autologous bone marrow to rescue rhesus monkeys from acute graft-versus-host disease. Exp. Hemat. 22: 32, 1972.

8) Graw, R.G., Jr., Yankee, R.A., Rogentine, G.N., Leventhal, B.G., Herzig, G.P., Halterman, R.H., Merritt, C.B., McGinniss, M.H., Krueger, G.R.D., Whang-Peng, J., Carolla, R.L., Gullion, D.S., Lippman, M.E., Gralnick, H.R., Berard, C.W., Terasaki, P.I., and Henderson, E.S.: Bone marrow transplantation from HL-A matched donors to patients with acute leukemia: Toxicity and antileukemic effect. Transplantation (In press).

9) Halterman, R.H., Graw, R.G., Jr., Leventhal, B.G., Johnson, G., House, S., Krueger, G.R.: Reactive lymphocyte blastogenesis during graft-versus-host reaction. Transplantation (In press).

10) Halterman, R.H., Graw, R.G., Jr., Fuccillo, D.A., and Leventhal, B.G.: Immunocompetence following allogeneic bone marrow transplantation in man. Transplantation (In press).

11) Peck, G., Herzig, G.P., and Elias, P.M.: Toxic Epidermal Necrolysis in a patient with graft-versus-host reaction. Arch. Derm. (In press).

Serial No. NCI-6302
1. Hematology-Supportive Care Branch
2. Clinical Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Tumor Immunology and Immunotherapy

Previous Serial Number: None

Principal Investigators: B. G. Leventhal, M.D., R. Halterman, M.D.

Other Investigators: D. Keim, M.D., A. LePourhiet, M.D., R. G. Graw, Jr., M.D., G. Herzig, M.D., E. S. Henderson, M.D., R. A. Yankee, M.D., R. Herberman, M.D., E. Rosenberg, M.D., D. Mann, M.D., Richard Simon, Ph.D., M. Lippman, M.D., G. Johnson, M. J. Sutherland

Cooperating Units: Immunology Branch, NCI; Office of the Associate Scientific Director for Program, C, NCI

Man Years:

Total: 10
Professional: 6
Other: 4

Project Description:

Objectives:

1. To obtain and store leukemia blast cells for immunologic, metabolic, and pharmacologic studies. (4X2)

2. To assess the immunologic reactivity of patients and their siblings to leukemia cells.

3. To assess the effect of chemotherapy on specific (i.e., antitumor) and nonspecific immune reactivity. (7XX)

4. To assess the effect of immunotherapy on the immune system and on the course of the disease. (7XX)(6XI)

5. To isolate, purify and characterize leukemia antigens.

Major Findings:

A. Storage of Leukemia Blast Cells.

Approximately 200 different specimens of leukemia, lymphoma and normal lymphocytes have been collected and stored with 10% DMSO in liquid nitrogen. These cells are being used in the following experiments:

1) As reagents in the immunologic studies to be discussed in greater detail below;

2) For the isolation and study of RNA dependent DNA synthetase and to evaluate the pharmacologic effect of rifamycin derivatives in the laboratory of Dr. R. Gallo and his coworkers.

3) For the detection and characterization of a steroid binding protein by Dr. M. Lippman in the Laboratory of Dr. E. B. Thompson.

In this latter study, specific cytoplasmic and nuclear binding sites for steroid hormones have been found in leukemic blast cells. Preliminary data shows that lymphoblasts have high numbers of binding sites when first seen but as drug resistance develops these sites are no longer detectable. Certain myeloblastic cells have binding sites but or lower specificity. We are currently evaluating the clinical significance of these data.

Conclusions: Stored leukemia blast cells are a valuable resource. They are being used in many laboratories, including our own, for reagents, particularly in immunologic and pharmacologic studies.

B. Immunologic Evaluation of Patients' Reaction to Autologous Tumor.

Twenty patients with acute leukemia were studied in 3 assays, the mixed leukocyte culture (MLC), lymphocyte cytotoxicity assay (LCA), and skin tests, for evidence of immune reactivity against their own leukemia blast cells. All but one showed a positive response in at least one assay, with MLC positive in 17/20, in 8/16 and skin test in 13/18 patients at some point in the course of their disease. Skin test reactivity seemed to correlate with disease state, being positive 14 of 18 times when performed in remission and only 3 of 8 times in relapse. Positive reactions in the MLC tended to be most common 10-20 days after chemotherapy had been discontinued. The tests did not correlate well with one another and this lack of correlation is being investigated.

To more precisely define the specificity of such immunological reactions to leukemia cells, studies were performed using leukemia patients with identical twins and with HL-A matched siblings. After the patients were in remission, blood was drawn from the patients and their siblings to test for reactivity to the stored patient blast cells in MLC. It was

found that the patients reacted to autologous blast cells while the siblings did not. This finding strongly suggests that the reaction of patients to their own blast cells is specific and immune in nature rather than the result of some nonspecific mitogenic factor released during the processing of these cells.

Conclusion: The majority of acute leukemia patients show evidence of cellular reactivity to their own blast cells while their HL-A identical siblings do not react, giving evidence that this reactivity is specific.

C. The Effect of Chemotherapy on Reactivity to Autologous Tumor.

As mentioned above, when the results of the MLC in 20 patients was considered relative to time off therapy, it was seen that 100% of tests performed 15-20 days after treatment were positive with a lower percentage of positive tests if the test was performed either closer to, or farther away from treatment. These observations were extended in 9 individual patients with a battery of antigens including autologous tumor in four. In all the patients the augmentation effect of intermittent systemic chemotherapy on *in vitro* reactivity was seen both to general antigens, and to autologous tumor cells.

Conclusion: It is possible to augment the *in vitro* reactivity of patients to their own tumor cells with properly timed chemotherapy.

D. Immunotherapy

Patients with previously treated ALL in relapse were given asparaginase, 15,000 IU/m^2/day for 28 days, either alone or in combination with 5-day courses of actinomycin D, 0.4 mg/m^2/day. Complete remission was achieved in 8/10 patients on asparaginase alone and 7/8 on the combination. At remission, patients were randomized to therapy with 1) Pasteur Institute BCG, 75 mg, freeze-dried weekly for 4 weeks by scarification plus cell inoculation, or 2) monthly courses of methotrexate (MTX), 15 mg/m^2 for 5 days followed in 10 days by cell inoculations every other day x 4. All cell inoculations were intradermal with $4x10^7$ leukemic cells from a single allogeneic donor per patient. Median remission duration of 7 patients on BCG was 93 days (range 64-216) and the median remission on the 6 patients receiving MTX was 97 days (range 67+-203+). Median remission duration on a previous asparaginase protocol was 64 days. The skin reactions of these patients were tested to PPD, SKSD, mumps, candidin and leukemic blast cell membrane. All patients but 2 had at least 1 positive skin test before therapy but all were tuberculin negative. Of those on BCG, 6/6 developed positive skin reactions to tuberculin, and 1/6 on MTX. In each group, 2/6 patients showed a definitely positive skin test (more than 5 mm induration), to the immunizing cell membrane preparation after therapy. The median remission duration in 6 patients showing no evidence

of delayed skin hypersensitivity after therapy with the immunizing cell preparation was 72 days (range 64-103) while in the group who showed some induration on skin testing (even if less than 5 mm.) the remission duration was 178 days (range 93-216). This demonstrated again that immunologic reactivity may be correlated with remission duration. Lymphocyte transformation in vitro was measured to PPD, SLO, smallpox, candida, PHA and allogeneic cells; 6/6 BCG treated patients showed an increase in reactivity to PPD ranging from 3-20 times baseline (median 14). MTX treated patients also showed a slight increase in reactivity to PPD ranging from 1-6 times baseline (median 2.5) presumably the effect of repeated skin tests. The response to immunizing blast cells went up in only two of the BCG treated patients and fell strikingly in two others. The response to immunizing blast cells rose in 4/6 MTX treated patients and fell in one. There was no consistent change in the PHA response. Thus the patients getting BCG and cells showed a specific increase in response to tuberculin both in vivo and in vitro but were no more reactive to the immunizing cell preparations than the patients getting MTX and cells.

As the study progressed and it became clear that we were not seeing an adjuvant effect with BCG, we increased the dose and gave the BCG intradermally instead of by scarification. Using the intradermal method we have had ulceration of the lesions and two patients on this protocol have been started on INH and ethambutol while they await healing of ulcers 2 and 5 cm. in diameter. We have tested patient sera for antibody to HL-A antigens and have not seen these antibodies develop during the early stages of this study. We feel therefore that the degree of alloimmunization is low.

Conclusion. BCG did not show a general adjuvant effect on the cellular immune response, although we did produce specific immunity to tuberculin. MTX augmented the responses to all antigens in a cyclic fashion. Immunotherapy using leukemia blast cells requires technical expertise in cryogenics and cell culture. The cells are non toxic and the degree of alloimmunization is small. There is to date no evidence that this therapy affected the clinical course of the disease.

E. Development of Assays for Non Lymphocyte Components of the Immune Response.

We realize that to date, in assaying delayed hypersensitivity and in vitro response to antigens we have looked only at the response of small lymphocytes in the immune system. We are in the process of measuring antibody response as well, both to tumor and to other antigenic stimulation. We have also attempted to measure the effect of chemotherapy on granulocyte function with NBT tests, and indeed, in a small number of patients it appeared that the capacity of the granulocytes to respond to stimulation in vitro with endotoxin was not diminished after a week of POMP therapy. We are planning to develop assays for macrophage function

in our patients as well as to round out our immunologic assay profile. A contract has been proposed which might allow us to have our standard lymphocyte studies done outside NCI leaving us more time to work on developing new methods.

Conclusion: Assays for other components of the immune response, in addition to small lymphocyte function, are being developed.

F. Effect of Bone Marrow Grafting on Immune Reactivity in General and to Autologous Tumor.

Four patients who had had bone marrow grafts performed during the past two years had their immune status after grafting evaluated using skin tests, response to in vitro antigen, and antibody response. It was found that in general, cellular immune responses were depressed more strikingly than humoral responses, and that the degree of suppression tended to be proportional to the degree of severity of GVHR. One other patient was followed in detail after grafting. He developed reaction to lymphocytes, spontaneously, during the course of GVHR, whose presence could be detected by their uptake of H^3TdR after 2 hours of incubation, as well as by pyronin methyl green staining. He was also found to have developed reactivity to autologous blast cells after grafting. It is not clear whether this reaction of circulating cells in the recipient represents graft-versus-normal tissue elements or graft-versus-tumor reactivity.

Conclusion: "Adoptive" immunotherapy for leukemia has been attempted with bone marrow grafts. There is evidence that the bone marrow chimeras are immunosuppressed, particularly as regards cellular immune reactivity. They were, however, able to survive documented viral infections. Graft-versus-blast cell activity was demonstrated in one patient but the implications of this reactivity are not clear.

G. Characterization of Tumor Antigens.

Dr. Dean Mann, of the Immunology Branch, NCI, has developed antisera to a soluble membrane antigen from the Burkitt lymphoma tissue culture cell in RAJI. These antisera were first shown to be cytotoxic to cells from 8 of 15 leukemia patients in relapse but not to cells from 527 normal donors. Further study in 9 patients followed serially showed that a positive reaction to these antisera was found only in patients in bone marrow relapse and that reactivity disappeared when the bone marrow was in remission. Reactivity to both myelogenous and lymphatic leukemia cells is present in these antisera. This is an exciting development sice it suggests that a common tumor, or tumor-associated antigen, may be present on the surface of acute leukemia and RAJI cells. These antisera may be useful for definition of complete remission by peripheral blood testing. These antisera, or RAJI cells themselves, may also be useful in immunotherapy.

Conclusion: A common tumor or tumor-associated antigen may be present on the surface of RAJI and of acute leukemia cells.

Significance to the Program:

Our data, when added to those of others, make it clear that human tumor antigens do exist and that the majority of patients are reactive to their own leukemia cells either in vivo or in vitro. These reactions have been shown to be immunologically specific in that they do not occur in HL-A matched siblings. The nature of these antigens is not clear, but it is exciting that there may be a common antigen on the surface of the RAJI and the leukemia cell, as detected by a heterologous antiserum. To date, immonotherapy trials with BCG and allogeneic blast cells have shown the development of specific immunity to tuberculin, but no adjuvant effect of the BCG on cellular immune response. We have demonstrated, however, that the in vitro immune reactivity of patients against their own tumor cells can be augmented during chemotherapy and hope that this knowledge will allow us to design chemotherapy protocols with maximal tumor cell kill and minimal immunosuppression.

Proposed Course of the Project:

The main emphasis of this program in the next year will be a continued analysis of immune reactivity of patients to their own tumors, including the evaluation of aspects of the immune response other than cellular immune reactivity, such as cytotoxic and blocking antibody, and macrophage reactivity. Possible cross-reactivity of patient and RAJI antigens will also be studied. The search for new materials with adjuvant activity will be continued. In addition to our own studies, a contract has been funded with Dr. David Weiss, in Israel, to investigate the effect on the immune system of the methanol extractable residue of BCG, called MER. We hope to eventually be able to manipulate the immune response to tumor antigens with a judicious combination of chemotherapy and immunotherapy.

Honors and Awards:

Brigid G. Leventhal, M.D.

1. Immunotherapy of Acute Leukemia. Presented at the Cancer Conference, Melbourne, Australia, March 1972.

2. Histochemistry in Clinical Hematology, Faculty of Histopathology, Armed Forces Institute of Pathology, Washington, D. C., January 1972.

Roger H. Halterman, M.D.

1. Detection of a Leukemic-specific Antigen. Presented at the American Cancer Society Science Writers' Seminar, Florida, March 1972.

2. Immunology and Immunotherapy of Leukemia. Presented at Raleigh Academy of Medicine, Raleigh, N. C., October 1971.

Publications:

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Serial Number NCI 3024
1. NCI-VA Medical Oncology Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report

July 1, 1971 through June 30, 1972

Project Title: Chemotherapy of Cancer

Previous Serial Number: 3024

Principal Investigator: Oleg S. Selawry

Other Investigators: Martin H. Cohen, Patrick J. Creaven, Heine H. Hansen, Franco M. Muggia, Ronald L. Stephens

Cooperating Units: The NCI-VA Medical Oncology Branch represents a joint effort of the National Cancer Institute and the Veterans Administration. Funds are provided by the National Cancer Institute, administered by the Veterans Administration Central Office. Facilities are provided by the Veterans Administration Hospital in Washington, D. C.

Man Years:

Total:	43 3/4
Professional:	22 3/4
Other:	21

Project Description:

Objectives:

1. Improvement of treatment for patients with bronchogenic carcinoma.

2. Introduction of new anticancer agents into clinical use and pharmacology of new antitumor agents.

3. Improved use of chemotherapeutic agents of established value.

Summary of Major Developments:

1. A feasibility study on intensive therapy of patients with inoperable bronchogenic carcinoma was completed. A median survival of 13 months for patients with limited disease (2-3 times the expected median survival) was encouraging and led to a comparative clinical trial, including 80 patients from this branch.

Our efforts were amplified through the establishment of a working party for therapy of lung cancer, including surgeons, radiotherapists, chemotherapists, thoracologists, immunologists, pathologists and a biostatistician from a small number of major institutions. Work proceeds according to a master plan, based on review of available data, with advancement of treatment plans adjusted to the biological characteristics of the 4 major types of lung cancer.

Special efforts were made toward better staging (bone marrow examination, peritoneoscopy, brain scan, gallium scan), better quantitation of response (fiberoptic bronchoscopy, hormone assays) better knowledge of cell kinetics (highest labelling index for small cell carcinoma) and better supportive care (laminar air flow - reverse isolation room).

2. Three new agents received initial clinical trial, five studies were conducted to explore more effective use of agents of recognized efficacy. One, CCNU, was compared with BCNU in patients with advanced Hodgkin's disease, showing significant superiority of CCNU over BCNU in a cooperative study of ALGB.

3. Five agents received extensive study of pharmacokinetics.

4. In these efforts, 356 patients were seen in consultation and 336 new patients were admitted to the branch, including 253 patients with bronchogenic carcinoma.

5. Experience gained is reflected in 20 papers, 8 abstracts, and 33 lectures and seminars given by the staff.

Detailed Reports:

1. Clinical Research

1.1 Improvement of treatment for patients with bronchogenic carcinoma. Oleg S. Selawry, Heine H. Hansen

An interdisciplinary working party for therapy of bronchogenic carcinoma was established including the Mayo Clinic, M.D. Anderson Hospital and Tumor Institute, University of Louisville School of Medicine, Louisville, Ky., University of Capetown, South Africa, and the Hospitalier Universitaire Lariboisiere, Paris, France with statistical support through Dr. Marvin Zelen. Two meetings have been held, leading to agreement on reporting, criteria for microscopic classification and staging, and development of a protocol for small cell carcinoma.

1.11 Protocol for Intensive Chemotherapy Plus Radiotherapy in Patients with Bronchogenic Carcinoma (ST 42). Heine H. Hansen, Oleg S. Selawry

This treatment program represented a pilot study to explore the feasibility of intensive therapy for patients with inoperable bronchogenic carcinoma. All patients received

chemotherapy with pairs of drugs including a cell cycle stage sensitive and a cell cycle stage non-sensitive agent in sequence of expected response. Drugs were given to individual tolerance for the duration of response. Subsequent pairs of drugs were given in the presence of progressive disease. Patients with disease limited to one hemithorax also received radiotherapy (5000 R in 6 weeks), added 3 weeks after institution of chemotherapy.

The study was initiated in April, 1969. Forty-six patients were entered on the study, forty-four evaluable patients, 27 with limited disease, 17 with extensive disease. Of the 24 patients evaluable for tumor response during Phase I of the study (chemotherapy with Cytoxan and methotrexate), 5 had objective response; 14, static disease; and 5, progressive disease. Phase II of the study (chemotherapy with dactinomycin and vincristine in combination with radiotherapy) produced 6 with objective response in 19 evaluable patients while 10 patients had static disease and 3, progressive disease. The data from the study indicate that the median survival for patients with limited disease was 13.2 months and 4.0 months for extensive disease. These data are highly favorable when compared with historical controls. The response rate and survival data for patients with small cell carcinoma, adenocarcinoma and large cell carcinoma of the lung were unexpectedly favorable.

Disposition: The study was discontinued in April, 1972. The experience gained served for the design of our current study (1.12).

Ref.: 4.51, 4.53, 4.69, 4.6.15, 5.5, 5.7

1.12 Comparative Treatment Program for Intensive Chemotherapy and Radiotherapy in Patients with Inoperable Bronchogenic Carcinoma (ST 53). Heine H. Hansen, Oleg S. Selawry

This study was initiated in September, 1970; patients staged as limited disease are randomized to either radiotherapy alone or radiotherapy plus chemotherapy. Patients with extensive disease are randomized either to a 2-drug combination chemotherapy program or a 3-drug combination program. In the past year 84 patients from this Branch have been included into the study. Other cooperating institutions include the Groote Schuur Hospital, Capetown, South Africa (49 patients), the University of Louisville Medical Center, Louisville, Kentucky (8 patients), and the Mayo Clinic, Rochester, Minn. (3 patients). Seventy-nine patients had limited disease, 69 had extensive disease at time of inclusion into the study. A detailed analysis of the data is in progress. Preliminary analysis suggests superiority of a 3-drug combination chemotherapy in small cell carcinoma as compared to the 2-drug combination in extensive disease. An unusually high incidence of brain

metastases has been observed, suggesting improved extracranial control of the disease.

Disposition: The study continues. Based on these results, a new protocol for the treatment of small cell carcinoma has been designed, including elective brain irradiation and extensive radiation to the abdomen to be initated upon completion of the present study.

1.13 Bronchoscopy and bacteriology of the lower respiratory tract in patients with bronchogenic carcinoma. Martin H. Cohen

One of the major impediments of intensive therapy is the insensitivity of methods to recognize progressive disease. Serial fiberoptic bronchoscopy is used as additional means to evaluate tumor size and to obtain tumor cells. Evaluation of the microbial flora (and proper antibiotic therapy) might represent an added advantage. The Olympus flexible fiberoptic bronchoscope of 5 mm diameter has been acquired and serial examinations have been initiated. It is honed that serial cytology and biopsy of accessible bronchogenic tumors will allow a more rapid determination when a given tumor is no longer sensitive to the chemotherapeutic drugs being used so that the treatment regimen may be altered. With regard to bacteriology, knowledge of the flora of the lower respiratory tract in advance of clinical function will allow better selection of appropriate antibiotics.

1.14 Laminar Air Flow Reverse Isolation Project. Oleg S. Selawry, Joseph Fedorko, Anita Tvardzik and Medical Staff

Two laminar air flow reverse isolation units were installed for participation in evaluation of reverse isolation for the purpose of intensive chemotherapy of malignant disease. Five patients have been treated in the units for a total of 136 days, as of 4/10/72. Three patients with acute myelocytic leukemia have been treated according to ALB #7122 in the units. Complete remission was achieved in two patients, after 57 and 42 days treatment in the units; one patient is currently being treated, in his 38th day in the unit. One patient with Kaposi's sarcoma expired after 24 days in the unit. One patient in the acute phase of chronic myelocytic leukemia expired after 26 days treatment in the unit.

Initiation of a controlled clinical trial with patients with bronchogenic carcinoma is imminent.

Strict isolation procedures have been observed. Microbiological monitoring included weekly culturing of the patient and his environment. During this fiscal year 1,200 patient cultures were taken for bacterial and fungal studies and 100 cultures were sent out for virus studies, including serum samples for viral serology. About 4000 cultures were taken of surfaces within the units by the RODAC method, 500 by the swab method, and 300 evaluations of water were made. Monitoring revealed

that the patients' oral cavity and intestines were the most difficult areas to maintain in a sterile condition. In the room the sink and shower drains and the toilet were most difficult to maintain in sterile limits, with Pseudomonas aeruginosa being most prevalent.

Ref.: 4.11

1.15 Improvement of staging. Heine H. Hansen, Franco M. Muggia

1.151 Osseous system

a. The superiority of bone marrow examination and ^{85}Sr bone scan as compared to bone survey has been further substantiated. In 150 consecutive cases studied, the incidence of bone marrow invasion for small cell carcinoma was 45%; for adenocarcinoma, 18.5%; for large cell anaplastic carcinoma, 14%; and for epidermoid carcinoma, 2.5%.

Ref.: 4.32, 4.33, 5.6

b. The occurrence of osteoblastic metastasis in small cell carcinoma and adenocarcinoma has been established in 7 and 2 patients, respectively, a phenomenon which has not been previously recognized.

Ref.: 4.34, 5.11

c. Hormonal studies to clarify the pathogenesis of the occurrence of osteoblastic and osteolytic lesions is under investigation, in collaboration with Dr. A. H. Tashjan of the Harvard School of Dental Medicine.

1.152 Hepatic system

Peritoneoscopy and liver biopsy for detection of hepatic metastases have been performed in 65 patients. Metastatic lesions in 5 patients with small cell carcinoma and one patient with epidermoid carcinoma have been documented. Correlation with liver scans and biochemical parameters appears to be of limited value. Dr. N. Trujillo acted as consultant.

1.153 CNS system

Brain scanning appears to be of limited value. Therefore, the evaluation of routine cerebrospinal fluid evaluation with cytocentrifuge cytology and LDH-isoenzymes has been initiated.

1.154 Other Staging Procedures

a. Hormonal screening: Collaboration with Dr. Saul Rosen of the National Cancer Institute has continued with 100 cases evaluated for HCG and HCS levels. Positive findings have occurred in 4 patients. In addition, thyroid function tests have been carried out.

b. α-Fetoprotein has been measured in 100 patients with bronchogenic carcinoma by R. McIntire, NIH. In one patient, elevated levels were documented.

c. Pretreatment and post-treatment serum zinc levels have been obtained in 75 patients, in cooperation with the Trace Elements Research Unit, Chief, Dr. James Smith, Washington VA Hospital. No correlation to diagnosis or objective response was noted.

Ref.: 5.18

1.16 Cell kinetic studies. Franco M. Muggia, Ronald L. Stephens, Susan Oster

Data has been obtained in the cell kinetic studies on the labelling index, DNA synthetic time (t_s), generation time, and growth fraction in patients with untreated bronchogenic carcinoma. Suitable patients have been given local thymidine injections followed by aspiration, punch or needle biopsies. Specimens have been processed by standard radioautographic techniques and mitotic index, labelling index, percent labelle mitosis determined, and duration of DNA synthesis by the double label technique. In addition, the increase of the labelling index with intratumoral injection q 12 hours is being studied. The recent use of the peritoneoscope in staging of patients has permitted a beginning in the study of the liver as a metastatic site. Twenty-one patients with various types of cancer involving the bone marrow have been studied. Eight patients with small cell carcinoma of the lung have had labelling indices done on tumor nodules. Of the 8 patients, who had received no prior chemotherapy, the LI (labelling index) was 8-24% (median, 17%). The LI in one patient was 19, 7, and 10% at onset, six and ten months of treatment. To estimate the proliferating fraction, a tumor nodule in an untreated patient was injected with ^{3}HTdR at approximate 8-hour intervals. The LI showed an increase from 24 to 39 and to 54% at times 0, 24, and 46 hours, respectively. The genera high LI after single and multiple pulses of ^{3}HTdR suggests a relatively large growth fraction in this tumor. The latter

may be a factor in determining the frequent marked regression of small cell carcinoma observed following initial therapy.
Disposition: Studies will be intensified and will be extended to preoperative labelling.

Ref.: 4.55, 4.58

1.2 Initial clinical trial of new anticancer agents.

1.21 Phase I and Phase II studies of CCNU (1-(2-chloroethyl)-3-cyclohexyl nitrosourea (ST 29). Heine H. Hansen

The study continues; 31 patients have been exposed to CCNU at a dose level of 130 mg/m^2 initially. A dose schedule of 130 mg/m^2 q 4 weeks appears feasible and has been explored in 15 patients. Therapeutic results have been seen in 3 patients with gastrointestinal carcinoma, 1 patient with reticulum cell sarcoma, and 1 patient with head and neck cancer. At repeat dosage (>3) cumulative hematologic toxicity is noted which can be easily compensated for by regulating the dose in accordance to individual tolerance.
Disposition: The studies will continue until 6 patients had 6 or more consecutive doses.

Ref.: 4.31

1.22 Treatment of unresectable bronchogenic carcinoma with bleomycin and streptozotocin (ST 62). Heine H. Hansen

The study was initiated for the evaluation of tolerance and antineoplastic effect of a combination of two non-myelosuppressive agents in patients with lung cancer bleomycin was given at a dose of 30 mg/m^2/wk i.v., streptozotocin at a starting dose of 7000 mg/m^2/wk i.v. for 6 weeks. One of these, bleomycin, has been reported to have activity when used alone. Eight patients have been admitted to the study. No objective response has yet been noted.
Disposition: The study will continue.

1.23 Protocol for the initial clinical trial of isophosphamide (ST 54) Martin H. Cohen

In the Phase I study doses were started at 200 mg/m^2 based on animal toxicity data. Currently a dose level of 3900 mg/m^2 has been reached. No toxicity was noted at doses between 200-700 mg/m^2. At 1000 mg/m^2, one of three patients had a fall in white blood cell count to 4600/mm^3 on day 9. No toxicity was noted at 1300 and 1700 mg/m^2. Starting at 2200 mg/m^2 most patients had nausea with occasional vomiting on the

day of treatment. Dypsnea and mild hematuria were noted in 1/3 of the patients at doses between 2200 and 3900 mg/m^2. Leukopenia to 3500/mm^3 was seen in 1 patient at 3900 mg/m^2. There have been no thrombocytopenia or BUN elevations thus far. No liver function abnormalities could be definitely related to drug treatment. In no case has cystitis and hematuria been serious enough to require bladder irrigation with sulfhydryl compounds.
Disposition: The study will continue.

1.24 Phase I evaluation of BPP (±bis-(3,5 dioxopiperazin-1-yl) propane. Patrick J. Creaven

Eighteen valid patients have been entered into the study at doses ranging from 1.0 to 10.5 g/m^2 as a single oral dose. Only mild toxicity was noted. Toxicity was hematologic and included leukopenia in 13 patients and thrombocytopenia in 1 patient. Toxicity was noted at 3.3 g/m^2 (3/5 pts.), 4.4 g/m^2 (1/2 pts.), 5.9 g/m^2 (3/5 pts.), and 7.9 g/m^2 (6/8 pts.). Exploration of 10.5 g/m^2 was begun but since 1/3 patients was not toxic at this dose it was decided to proceed to a dose of 3.0 g/m^2/wk x 6, 3 patients to receive a single weekly dose and 3 to receive a split (t.i.d.) weekly dose. This evaluation is in progress.
Disposition: The study continues.

Ref.: 4.22

1.25 Protocol for initial clinical trial of epipodophyllotoxin derivative (EPEG) ST 64

The objective of this study is to determine the clinical pharmacology and toxicology of the compound in patients with cancer, unresponsive to therapy of established value. Initial dosage is determined from preclinical trials; the drug is given intravenously, weekly, for six weeks. EPEG is unique since it is the most active among the podophyllum liganes against a spectrum of experimental animal tumors and the most active plant product tested by the NCI against L1210. To date, two patients have been treated at the initial dosage of 20 mg/m^2 weekly i.v. for six weeks. One patient is evaluable for toxicity; he developed consistent leukopenia with moderate severity following each weekly dosage. The other patient developed hypotension.
Disposition: The study will continue.

1.3 Improved use of chemotherapeutic agents of established value. Heine H. Hansen, Oleg S. Selawry, Patrick J. Creaven

1.31 Methotrexate-leukovorin Phase I and Phase II study

Three additional patients have been studied, including ^{3}H-methotrexate pharmacologic studies in all three patients. The optimal ratio of methotrexate to leukovorin has been established to be 6:1 with an interval of 72 hours for compensation.
Disposition: The study will continue, using ^{3}H-methotrexate.

1.4 Participation in Cooperative Studies

Dr. Martin Cohen assumed responsibility as Assistant Executive Officer of the ECOG and is intramurally responsible for all ECOG studies. Dr. Heine Hansen is intramurally responsible for all ALGB studies and maintains liaison with ALGB.

1.41 ST 7, Hodgkin's disease: Non-randomized patients. One patient entered with reticulum cell sarcoma.

1.42 Lymphocytic lymphoma (ST 20): One patient entered with poorly differentiated reticulum cell sarcoma.

1.43 Evaluation of IV Procarbazine (Matulane) (ST 50), Solid Tumor Service: Two patients entered, both with lung cancer (1 anaplastic, 1 adenocarcinoma).

1.44 Comparison of Intermittent Cytosine Arabinose Plus Thioguanine with Cytosine Arabinose Plus CCNU in Induction and Maintenance of Untreated Acute Myelocytic Leukemia (Protocol ALGB 7121). Three patients entered.

1.45 Comparison of Intermittent Cytosine Arabinose Plus Cyclophosphamide with Cytosine Arabinose Plus Daunorubicin in Induction and Maintenance of Untreated Acute Myelocytic Leukemia (ALGB #7122). Two patients entered.

1.46 Intermittent Prednisone with or without Chlorambucil Given Continuously or Intermittently in the Treatment of Stage III or IV Chronic Lymphocytic Leukemia (ALGB #7133). Acute Leukemia Group B. One patient entered.

1.47 Actinomycin - D and Mithramycin in Hypercalcemia (EST #0270) Eastern Cooperative Oncology Group. Three patients entered, two with lung cancer, and 1 with cancer of esophagus.

2. Laboratory Research

2.1 Pharmacology of New Antitumor Agents. Patrick J. Creaven, Larry M. Allen

2.11 Study of plasma levels of camptothecin (CS) and the influence of μmc flow rates on CS excretion and toxicity.

Minimum plasma levels during each 24 h of a daily x 5 course of CS were determined using a fluorometric method devised in this laboratory. Twelve complete courses were studied in patients with normal renal function. Mean C_{min} values ranged from 0.17-1.23 μg/ml and showed essentially no correlation wit dose (6.25-20 mg/m^2/day x 5 (r=0.16). Correlation of mean C_{mi} values with hematological toxicity (WBC<$10^5$and plat.<10^5) was fair in 13 evaluable patients. Of 8 courses resulting in no toxicity, one mean C_{min} value was >0.5 ug/ml; of 5 courses resulting in hematologic toxicity, one was <0.5 μg/ml. Of 7 receiving 15 or 20 mg/m^2/day x 5 of CS in whom daily urine volumes were measured, 4 whose mean urine volume was 568 ml/day developed hematologic toxicity, and 3 whose mean urine volume was 1929 ml/day did not (P<0.01). CS is highly bound to serum albumin (SA); equilibrium dialysis indicates a binding constant of 6 x 10^7 M^{-1} and a value for n of 0.8, but toxicity was unrelated to SA, total protein or the use of drugs which bind to SA.
To explain the relation between urine volume and toxicity of CS the plasma decay of CS after a single dose was studied. The plasma decay of CS after a single i.v. dose fits the equation for a biexponential decay $C = Ae^{-t}$ Be^{-Bt} (Eq. 1). The minimum plasma levels during each 24 h of a daily x 5 schedule (C_{min}) at doses from 6.25-20 mg/m^2 BSA/day predicted from Eq. 1 agree well with the observed values in 5/10 patient studied but deviated markedly in 5/10 patients. No evidence for metabolism of CS could be obtained in vivo or from in vitr microsomal enzyme systems using rat liver microsomes. The recovery of CS in the urine of two patients was 34% and 38% with urine volumes of <2L/24 h and 47% and 69% with urine volumes of >3L/24h. Uncorrected renal clearances of CS were 7.1 and 12.24 ml/min with urine flows of 49 and 52 ml/h and 41.5 and 43.7 ml/min with urine flows of 114 and 274 ml/h. The $t_{½}$ of the excretory phase of CS in one patient was 14.5 h when the urine flow rate was 67 ml/h and 8.25 h when the urine flow rate was 177 ml/hr. The results clearly indicate the dependence of CS clearance on nerve flow rate and may explain the valuable toxicity seen clinically with the drug.

Ref.: 4.21, 4.23, 4.24

2.12 Study of the blood and urine levels of isophosphamide (IP) following single i.v. doses.

Urinary levels of alkylating activity after a single dose of IP from 200 mg/m^{2}to 3800 mg/m^2 have been measured in 22 patients who have received the drug in the course of the Phase

I evaluation of this drug. Alkylating activity in plasma has also been measured. At low doses no measurable alkylating activity was found in the blood. At higher doses low levels were found. Urine alkylating activity reaches a peak in the first or second 8 h collection period and thereafter declines rapidly but at 72 h past dosage measurable levels are still present in the urine. Levels are lower than those seen with comparable doses of cyclophosphamide.

2.13 Study of the activation of Isophosphamide (IP) by rat liver microsomes.

The metabolism of IP was studied in rat liver homogenates. It was found that the principal site of liver activation is the microsomal fraction. This activation follows simple Michaelis-Menten enzyme kinetics, is NADPH dependent, is not inhibited by aniline, but is inhibited by ethylmorphine and 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525A). The Vmax for IP and CP were found to be the same (5.4 μ Moles HN2 equivalent/9 liver/h) but apparent Km for the activation of IP was about twice that for activation of CP. This is felt to be due to hindrance by the endocyclic chloroethyl group of the carbon atom which is the prime site for metabolic attack by microsomal hemaprotein in the case of CP.

Ref.: 4.27

2.14 Studies of the effect of isophosphamide (IP), phenobarbital (PB), and IP plus BP pretreatment on the activation by rat liver microsomes of IP and cyclophosphamide, the dealkylation of ethyl morphine (EM) and aminopyrine and the hydroxylation of aniline.

The effects of pretreatment with IP, PB and both together on the microsomal activation of IP, the dealkylation of EM aminopyrine, the hydroxylation of aniline and the levels of microsomal RNA and protein and nuclear DNA in rat liver were studied. PB given 55mg/Kg/day for 3 days increases the specific activity of microsomal metabolism of CP (140%) and IP (218%). Concomitant treatment with IP (44mg/Kg/day) for 3 days does not diminish the microsomal induction but leads to significant bladder toxicity (12/12 treated, 0/12 controls). Neither IP alone nor in combination with PB has any effect on the level of liver cell nuclear DNA or microsomal RNA. However there is some interaction ($P<0.05$) between IP and PB on the level of microsomal protein ordinarily induced by PB alone.

Ref.: 4.26

2.15 Influence of the activation of isophosphamide (IP) on the synthesis of DNA in L1210 cells.

The effect of the liver activation of IP on its ability to inhibit incorporation of ^{3}H thymidine into DNA of L1210 cells was studied. IP was effective in inhibiting incorporation only at high concentration, whereas incorporation was inhibited at much lower concentrations after microsomal activation. Microsomal activation enhanced the ability of ^{14}CIP to alkylate calf thymus DNA and this effect was increased 100% by PB pretreatment of animals over saline pretreated controls.

2.16 Study of the microsomal metabolism of thalicarpine (TC).

The demethylation of TC by rat liver microsomes is currently under study, using formaldehyde release in vitro as a method for determination of demethylation.
Disposition: The study continues.

2.17 Studies on the effect of thalicarpine on the metabolism of ethyl morphine and aniline.

The effect of TC in vitro on the demethylation of EM and the hydroxylation of AN is under study. Two enzymes are being used to determine KI for the inhibition of these activities by TC.
Disposition: The studies are being expanded to include the effect of TC in vivo on the microsomal metabolism.

2.18 Study of the effect of thalicarpine (TC) on the synthesis of DNA, RNA, and protein in L1210 cells.

The effect of TC on the incorporation of precursors into RNA, DNA, and protein of L1210 cells is currently under active investigation.

2.19 Study of the interaction of nitrogen mustard (HN2) with bovine (BSA) and human (HSA) serum albumin.

The interaction between alkylating agents and sulphydryl groups has been studied using HN2 as a model and alkylating agent BSA as a model cource of sulphydryl groups and dithiobisnitrobenzoic acid to titrate sulphydryl groups. The system was then used to assay alkylating activity from the products of the microsomal activation of IP. Fluorescence spectroscopy of BSA in the presence of HN2 revealed a decrease in relative fluorescence of BSA and an hypsochromic shift at λ_{EXC} of 287 nm. The possibilities of using this model for the assay of alkylating activity is being explored.

2.1.10 Study of gas chromatographic determination of isophosphamide (IP) in plasma.

A gas chromatographic method has been developed for IP and its usefulness in the estimation of plasma IP in man has been demonstrated.

2.1.11 Study of the plasma levels of methotrexate (MTX) following large i.v. doses.

The study of the dose-dependent pharmacokinetics of MTX, using high doses of MTX and restudying patients at maximum doses prior to leukovorin rescue, is being continued. Eight patients have been studied at 80 mg/m^2 i.v. and four of these have been re-studied at 2 x toxic dose after escalation of the dose by 50% increments every 2 weeks. The number of patients who have been restudied is still too small for any general conclusions.

2.2 Studies on the enhancement of the antitumor effect of bis-chloroethyl nitrosourea by membrane active drugs and caffeine. Martin H. Cohen

A continuing program is underway to study enhancement of the anti-tumor effect of bis-chloroethyl nitrosourea by various membrane active drugs and caffeine. Initial studies in this area were with vitamin A alcohol, which, when combined with BCNU and caffeine, produced 50% cures in leukemia L1210 as opposed to 5% cures with BCNU alone. The studies were then extended to evaluate whether membrane active psychotropic drugs could also enhance BCNU and caffeine effect. The most effective drug in this study was chlorpromazine which gave 66% cures. The dose of chlorpromazine used was 30 mg/m^2, a dose easily tolerable in human trials.
Disposition: Studies are continuing with the hope of determining the mechanism of action of these drugs by electron microscope and various biochemical studies. A Phase I protocol has been designed.

2.3 Studies in cell culture. Martin H. Cohen, Cleopatra Thornton

The cell culture laboratory is predominantly concerned with development of methods to determine optimal means of combining drug treatments to achieve maximal cell kill. For this purpose, two methods are being studied: 1) the Thayer-Himmelfarb spin culture system, which allows removal of drug from the system in an exponential manner, thus more closely approximating *in vivo* conditions, and 2) a method of fractionating cells in density gradients to separate cells that are destined to die following a drug treatment from those which have survived the treatment. The latter group of cells would then be treated again, either with the same agent or with a different drug, to determine the optimal timing of a second treatment.
Disposition: It is hoped to incorporate new drugs coming into clinical trial into this system. CTX studies will be done for all drugs to determine optimal methods of drug treatment.

2.4 Functions of the Hematology Laboratory. Heine H. Hansen, Brenda Shelton

The following tests were performed in the Hematology Laboratory during the time period 4/1/71-4/1/72:

Bone marrow aspirations	264
Bone marrow biopsies	272
"Touch-prep"	156
Differentials	58
Zinc levels	81
Liver biopsies "Touch-prep"	10
CSN evaluations (cytocentrifuge cytology)	26

Because of a staffing problem, the laboratory functioned to 100% capacity for only 9 months of the 12-month reporting period. The Model S coulter counter, provided by the Oncology Branch, in the General Laboratory of the VA HOspital, has been used for WBC and hematocrit tests on Oncology patients.

2.5 Pathology Laboratory Activities. Mary J. Matthews

As of January 1, 1972, Dr. Mary Matthews joined the staff as Senior Pathologist and Chairman of the Pathology Subcommittee of the Working Party for Therapy of Lung Cancer, in charge of joint review and distribution of all initial diagnostic slides of the WP-L on patients entering therapeutic trials for bronchogenic carcinoma. During the past year, cell blocks, smears, and filter preparations of approximately 1700 sputum specimens, bronchial washings, and/or other body fluids have been screened by the senior pathologist. Approximately 2000 surgical specimens, including materials submitted from other hospitals, have been interpreted or reviewed. Sixty autopsies of patients dying with neoplastic disease have been reviewed grossly and microscopically.
A cytotechnologist was added on 3/9/72 to prepare and screen all cytology specimens (approximately 250/month) and to set up duplicate staining techniques (Papanicolaŭ).

3. Additional Activities of the NCI-VA Medical Oncology Branch

3.1 Consultations

During the period April 1, 1971 to March 31, 1972, a total of 356 consultations were provided by the Oncology medical staff in the Washington VA Hospital, a 17% increase over the preceding year. In addition, numerous telephone calls by outside physicians concerning chemotherapy were answered.

3.2 Nursing (Ward and Ambulatory Care Clinic)

In October, 1971, 4 additional beds were opened, bringing the total to 26 ward patients. In November, 1971, the 2 beds in the Laminar Air Flow units were occupied, with staff using the opportunity to initiate sterile procedures gradually. By February, 1972, the ward reached full occupancy of 30 patients. Shortage in nursing staff has been alleviated since February, 1972. In July, 1971 there were 19 coworkers on the nursing staff, increasing to the April level of 33. The projected 41 was planned by June 1 for complete staffing for the ward, ambulatory clinics, and Laminar Air Flow units. The total number of patients treated during the one-year period April 1, 1971 to March 31, 1972 was 336, with the April total of 305. One Out-Patient Clinic was added, for a total of four per week. The total number of out-patients during the year was 170, with total number of out-patient clinic visits being 1737.

3.3 Social Work Service. Margaret Carolla

The social worker on the Medical Oncology Branch sees each patient on admission to the ward on an initial screening basis in order to ascertain what unmet needs exist and to anticipate problems needing her attention, both during and after the patient's hospital stay. Each patient is given an informal orientation to the ward. Supportive attention for the patient and family is sustained and/or intensified, as needed. The social worker operates as an active member of the treatment team, alert to the emotional and social aspects of the patient's continuing care. She acts as referring agent between patient and sources of financial assistance within the VA and in the community and assists each patient to meet the crisis of his disability in realistic ways. Coordination of efforts of intra- and extramural departments toward achievement of continuity of care (i.e. specialized home care and/or nursing home care) on an individualized basis is a responsibility of the social worker. Patients attending the Out-Patient Clinics are seen on a consultative basis, either through a physician referral or by request of the family. The VA Voluntary Service, at request of the Oncology Branch, provides coffee and cookies for the Clinic patients one afternoon a week, which helps provide a warmer atmosphere for the waiting patients and families. This service will increase to three times a week, as volunteer aid becomes available.

3.4 Dietetic Service. Sadie Arkwright

In July, 1971, one staff dietition and two dietary technicians were added to the Oncology staff. The dietition works closely with the other members of the medical team in providing total health care of the Oncology in-patients. Each patient is visited daily: Good

nutrition is stressed, nutrition histories and evaluations are prepared, pertinent information is documented on patients' charts, and diets are altered to meet individual needs. Dietary procedures have been formalized for the Laminar Air Flow units. The dietary technicians have been trained on this ward in established procedures of asceptic techniques. The "cooked food diet" was adopted for use in the Laminar Air Flow units in an attempt to lower the daily total count of bacteria via food. The dietition attends the regularly scheduled Oncology weekly rounds. Planned future activities include: food intake studies on selected patients, bacteria control studies, establishing dietary regimens in cooperation with out-patient staff to meet individual needs of patients in the Oncology Out-Patient Clinics.

3.5 Research Pharmacist. David Wolfson

The position of a research pharmacist-clinician has proven, in principle, very helpful in order to assure exact dosage of research drugs and to prepare agents under sterile conditions for patients in reverse isolation. Full development of the position toward that of a pharmacist-clinician is anticipated.

3.6 Educational Activities

Medical staff members attended and participated in regularly scheduled discussion meetings, rounds, and Tumor Board at the VA Hospital and the NCI. Informal discussions concerning research problems and developments in the field of oncology were held frequently. Staff physicians contributed 8 months of attending coverage on the VA Hospital medical wards.

Three trainees in medical oncology have completed one year; two rotating through the Solid Tumor Service and Leukemia Service for three months each, one spending the year at the Oncology Branch.

Fellows of the Cancer Therapy Evaluation Branch, NCI, rotated through the NCI-VA Medical Oncology Branch for 6 months per trainee.

Two George Washington University Medical School students spent one and two months on the Oncology ward. A Physical Diagnosis class was given instruction by Dr. Selawry.

Laboratory technicians attended lectures and seminars relating to their specific fields at the VA Hospital.

The dietician attended a dietetics course at Howard University.

Educational activities of the nursing staff during the past year related directly to care of the in- and out-patients. A major focus has been in-serivce training for nursing in the Laminar Air Flow units. The nurse in charge of the Laminar Air Flow nursing observed units and met with nursing staff of the units at the National Institutes of Health. In-serivce programs, both in the VA Hospital Nursing Service and on the Oncology ward, numbered over 65. An oncology staff nurse was appointed to the position of oncology nursing in-

structor who arranges for training programs and lectures and provides orientation of new nursing staff, over and above the regular hospital nursing orientation.

Four oncology nurses gave presentations at the months VA Hospital Nursing Service programs.

Seven graduate nursing students from Catholic University received clinical experience and training in oncology in the Oncology Branch for one month. In March, 1972, seven nurses of the Oncology Branch were cleared by the VA for i.v. administration and blood drawing, which alleviates the trainees'duties.

Three nurses attended a seminar on Nursing and Changing Responsibilities in Drug Administration.

The Head Nurse and Nurse-in-Charge of the Laminar Air Flow nursing visited the M.D. Anderson Hospital and Tumor Institute to observe procedures of the Laminar Air Flow units and general oncology nursing. The Nurse-in-Charge of Laminar Air Flow nursing visited the NCI and Baltimore CRC to observe Laminar Air Flow unit procedures.

Talks on various aspects of oncology--clinical research, laboratory research, total patient care--are presented at least once monthly by the head of the specific activity for all Oncology staff who can attend.

Presentations at the weekly comprehensive oncology rounds were given by members of the Oncology medical staff, staff of other departments of the Washington VA Hospital and other units of the National Cancer Institute, and outside specialists.

3.7 Training and Workshops Attended by Oncology Staff

S. Arkwright, Dietician, attended the annual meeting of the American Dietetic Association, Philadelphia, Pa., October, 1971; a Workshop of the American Hearth Association, Washington, D.C., March, 1972; a tour of the Life Island Unit, NIH, September, 1971.

Anita Tvardzik, RN, attended the LAF Working Group, April 25, 1972.

M.J. Bettis, Statistical Assistant, completed a course, Fortran IV at the NIH; attended a Workshop on Fundamentals of Biomedical Digital Computation, VA Hospital, West Haven, Conn., October, 1971.

M. Carrolla, Social Worker; J. Fedorko, Microbiologist; C. Thornton, Microbiologist; and E. Mackie, Administrative Assistant attended a one-day EEO training course, January, 1971.

4. Related Activities

4.1 Martin H. Cohen, M.D.

4.11 "Use of Laminar Flow Facilities," presented at the George Washington University Medical Center, Department of Oncolog and Hematology, January, 1972.

4.12 "Tumor Cell Kinetics in Acute Myelocytic Leukemia as Related to Current Treatment Regimens," presented at the

George Washington University Medical Center, Department of Oncology and Hematology, January, 1972.

4.13 "Enhancement of the Antitumor Effect of BCNU by Vitamin A and Caffeine," presented at the Annual Meeting of the American Association for Cancer Research. Proc. Amer. Ass. Cancer Res. 13:45, 1972.

4.14 Certified by the American Board of Internal Medicine, June, 1972.

4.15 Appointed Assistant Professor of Medicine, George Washington University School of Medicine, July, 1971.

4.16 Assistant Executive Officer, Eastern Cooperative Oncology Group, September, 1971.

4.2 Patrick J. Creaven, M.D., Ph.D.

4.21 "Studies on the New Antineoplastic Agents, Camptothecin and Isophosphamide," presented at a Seminar at George Washington University Medical Center, October, 1971.

4.22 "Clinical Introduction of New Anticancer Agent: ±bis-(3,5 dioxopiperazin-1-yl) Propane (BPP)," presented at the Phase, I, Phase II Liaison Meeting, March, 1972.

4.23 Creaven, P.J., and Allen, L.M. Camptothecin Plasma Levels during a 5-Day Course: Correlation with Toxicity. Proc. Amer. Ass. Cancer Res. 13:46, 1972.

4.24 Creaven, P.J. and Allen, L.M. Renal Clearance of Camptothecin Sodium in Man: Effect of Urine Volume. Proc. Vth Internat. Cong. Pharmacol. In press. (Abstract)

4.25 Allen, L.M., and Creaven, P.J. Metabolism of Isophosphamide by Rat Liver Microsomes. Proc. Acad. Pharm. Sci. In press. (Abstract)

4.26 Allen, L.M., and Creaven, P.J. Induction of Isophosphamide Metabolism by Phenobarbital. Proc. Vth Internat. Cong. Pharmacol. In press. (Abstract)

4.27 Appointed Special Lecturer in Pharmacology, Department of Pharmacology, George Washington University Medical Center, January, 1972.

4.3 Heine H. Hansen, M.D.

4.31 "CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea): A New Oral Nitrosourea," presented at the VIIth International Congress of Chemotherapy, Prague, Czechoslovakia, August, 1971. Abstracta: VIIth Internat. Cong. Chemotherapy B-8/8, 1971.

4.32 "Staging and Treatment of Patients with Bronchogenic Carcinoma," presented at the Oncology Conference, George Washington University Medical Center, October, 1971.

4.33 "A Comparison of BCNU and CCNU in Patients with Advanced Hodgkin's Disease," presented at the Phase I-II Liaison Meeting, National Cancer Institute, March, 1972.

4.34 "Experience with Bone Marrow Examination and Peritoneoscopy in the Staging of Patients with Non-Resectable Bronchogenic Carcinoma," presented at the National Conference on Lung Cancer, National Institutes of Health, Bethesda, Md., March, 1972.

4.35 Hansen, H.H., Muggia, F.M., and Napoli, L. Osteoblastic metastases in bronchogenic carcinoma. Amer. Soc. Clin. Oncology. No. 27, 1972. (Abstract)

4.36 Appointed Assistant Professor, Department of Oncology and Hematology, George Washington University School of Medicine, July, 1971.

4.37 Appointed Executive Officer, Working Party for Therapy of Lung Cancer, October, 1971.

4.38 Admitted to membership in the American Association for Clinical Research.

4.39 Participation in ALGB (co-chairman of Protocol for Comparison of CCNU and BCNU in Hodgkin's Disease), and ECOG.

4.4 Mary J. Matthews, M.D.

4.41 Executive Officer, Washington, Washington Society of Pathologists.

4.42 Program Director for the combined Annual Meeting of the Washington and Maryland Pathology Societies, Annapolis, Maryland.

4.43 "Pulmonary Calcinosis, Associated with Malignancies and Chronic Renal Disease," presented at the AFIP, February, 1972.

4.44 "Functional Acinar Cell Carcinoma of the Pancreas," presented at the Naval Medical Center, April, 1972.

4.45 Appointed Chairman, Pathology Subcommittee, Working Party for Therapy of Lung Cancer, October, 1971.

4.5 Franco M. Muggia, M.D.

4.51 "Staging in a Chemotherapy-Radiotherapy Protocol in Lung Cancer," presented at the Albert Einstein College of Medicine, New York, N.Y., July, 1971.

4.52 "Islet Cell Tumor," presented at Medicine Branch Rounds, NCI, September, 1971.

4.53 "Results of Staging and a Chemotherapy/Radiotherapy Protocol in Lung Cancer," presented at the Chemotherapy Conference, Memorial Hospital, New York, N.Y., September, 1971.

4.54 "Lung Cancer," presented at the Neoplastic Disease Conference, George Washington University Medical Center, September, 1971.

4.55 "Tumor Cell Kinetics," presented at the Hematology Seminar, George Washington University Medical Center, September, 1971.

4.56 "CNS Metastases from Bronchogenic Carcinoma," presented at the Medicine Branch Rounds, NCI, December, 1971.

4.57 "Staging and Treatment of Inoperable Bronchogenic Carcinoma," presented for the Pulmonary Service Seminar, Albert Einstein Hospital, December, 1971.

4.58 "Cell Kinetic Studies in Patients with Small Cell Carcinoma of the Lung," presented at the American Association for Cancer Research Annual Meeting, May, 1972. Proc. Amer. Ass. Cancer Res., 1972.

Oleg S. Selawry, M.D.

4.61 Moderator, Round Table Discussion on Chemotherapy of Acute Leukemia, XIIth Internation Congress of Pediatrics, Vienna Austria, August, 1971.

4.62 "Emergencies in Medical Oncology," Lecture presented at the VA Hospital, Washington, D.C., September, 1971.

4.63 "Efficacy of Bone Marrow Examination for Diagnosis and Staging of Patients with Cancer," presented at the Annual Meeting of the Kentucky Medical Association, Louisville, Kentucky, September, 1971.

4.64 "Preclinical Data of the Nitrosoureas,: presented at the meeting of the NACC, NIH, Bethesda, Md., September, 1971.

4.65 "Design and Goals of the Phase I Clinical Trail," presented at the Chemotherapy Conjoint Meeting on Preclinical Toxicology and its Clinical Correlation, Annapolis, Md., October, 1971.

4.66 "Chemotherapy and the Total Care of the Cancer Patient," presented for the VA Voluntary Service Advisory Committee VA Hospital, Washington, D.C., October, 1971.

4.67 "Reticulum Cell Sarcoma," presented at the Georgetown University Hospital Combined Tumor Board, Georgetown University, Washington, D.C., October, 1971.

4.68 Participant in Program of "New Protocol Design," VA Lung Group meeting, San Francisco, Calif., October, 1971.

4.69 "Pulmonary Carcinoma, Including Extrathoracic Manifestations," presented at the Walter Reed Army Hospital, Washington, D.C., October, 1971.

4.6.10 Participant in the Second Joint Working Conference of the NCI Chemotherapy Contracts Program, Annapolis, Md., November, 1971.

4.6.11 Chariman, First Meeting of the Working Party for Therapy of Lung Cancer, NIH, Behtesda, Md., November, 1971.

4.6.12 Participant in the Meeting of the Radiation Therapy Oncol Group, Denver,Colorado, December, 1971.

4.6.13 "Criteria of Response to Therapy," presented at the ECOG meeting, Atlanta, Georgia, January, 1972.

4.6.14 Chairman, Second Meeting of the Working Party for Therapy of Lung Cancer, NIH, Bethesda, Maryland, February, 1972.

4.6.15 "Response to Intensive Chemotherapy and Radiotherapy: Implications of Cell Type for Differential Therapy of Lung Cancer," presented at the meeting "Carcinoma of the Lung: A Status Report of the National Cancer Institute's Research," Bethesda, Md., March, 1972. Chairman, second day of the two-day meeting.

4.6.16 "Chemotherapy and Prospective Protocols," presented at a Cancer Workshop on Carcinoma of the Lung: Old Views and New Perspectives, WAshington University School of Medicine, St. Louis, Missouri, April, 1972.

4.6.17 Participant on a Panel Discussion "Death and Dying," Chaplains'Day Program, April, 1972.

4.6.18 "Superiority of CCNU (1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea; NSC 79037) over BCNU (1,3-bis(2-Chloroethyl)-1-Nitrosouear; NSC 409962) in Treatment of Advanced Hodgkin's Disease, " presented for the Acute Leukemia Group B at the Annual Meeting of the American Association for Cancer Research, Boston, Mass., May, 1972. Proc. Amer. Ass. Cancer Res., 1972.

4.6.19 "The Therapeutic Approach to Non-Resectable Lesions," presented at the Chemotherapy Conference: Course on Current Concepts in the Treatment of Malignant Disease, National Institutes of Health, Bethesda, Md., May, 1972.

4.6.20 "Respiratory Cancer," presented for the American College of Physicians Postgraduate Course: Perspectives in Cancer Medicine, Buffalo, New York, June, 1972.

4.6.21 Site Visits:
Mayo Clinic, Rochester, Minn.
VA Hospital, St. Louis, Missouri
Abraham Lincoln School of Medicine, Chicago, Ill.
Johns Hopkins University Medical Center, Baltimore, Md.
Cleveland General Hospital, Cleveland, Ohio

4.6.22 Membership and Participation:
American Association for Cancer Research
American Society of Clinical Oncology
NCI Operations Committee
NCI Decision Network Committee
NCI Experimental Design Committee
Veterans Administration Lung Group
Veterans Administration Project Review Committee
Acute Leukemia Group B, Chairman, Protocolon BCNU vs CCNU

4.6.23 Executive Officer ECOG

4.6.24 Chairman, Working Party for Therapy of Lung Cancer

5. Publications

5.1 Cohen, M.H. Enhancement of the antitumor effect of BCNU by vitamin A and caffeine. J. Nat. Cancer Institute. In press.

5.2 Cohen, M.H., and Carbone, P.P. Enhancement of the antitumor effect of certain alkylative agents by vitamin A. J. Nat. Cancer Institute. In press.

5.3 Cohen, M.H., Rotsztam, A., Shugoll, G.I., and Bowen, P.J. Electrocardiographic changes in acute pancreatitis resembling acute myocardial infarction. Amer. Heart J. 82:672-677, 1971.

5.4 Creaven, P.J., Burns, W.A., and Selawry, O.S. Widespread metastas and renal failure from carcinoma of the soft palate: Report of a case. J. Urol. In press.

5.5 Hansen, H.H., Muggia, F.M., Andrews, J.R., and Selawry, O.S. Intensive combined chemotherapy and radiotherapy in patients with non-resectable bronchogenic carcinoma. Cancer. In press.

5.6 Hansen, H.H., Muggia, F.M., and Selawry, O.S. Bone marrow examination in 100 consecutive patients with bronchogenic carcinoma. Lancet 2:443-445, 1971.

5.7 Hansen, H.H., and Selawry, O.S. Recent therapeutic developments in lung cancer. Med. Ann D.C. 40:580-582, 1971.

5.8 Heinemann, H.O., and Muggia, F.M. Hypercalcemia of Malignancy: Ethics of treatment. Ann. Inter. Med. 75:481, 1971.

5.9 Ho, B.T., Estevez, V., Tansey, L.W., Englert, L.F., Creaven, P.J., and McIsaac, W.M. Analogues of amphetamine. Studies of excretory metabolites of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) in rats. J. Med. Chem. 14:158, 1971.

5.10 Muggia, F.M., Creaven, P.J., Hansen, H.H., Cohen, M.H., and Selawry, O.S. Phase I clinical trail of weekly and daily schedule of camptothecin sodium (NSC 100880) and its correlation with preclinical studies. Cancer Chemother. Rep. In press.

5.11 Muggia, F.M., and Hansen, H.H. Osteoblastic metastases in small cell (oat cell) carcinoma of the lung. Cancer. In press.

5.12 Muggia, F.M., Selawry, O.S., and Hansen, H.H. Clinical studies with a new podophyllotoxin derivative 4' demethyl-epipodophyllotoxin-B-D-thenylidene glucoside. Cancer Chemother. Rep. 55:575-581, 1972.

5.13 Muggia, F.M., and Uktmann, J.E. Glomerulonephritis or nephrotic syndrome in malignant lymphoma, reticulum cell type. Lancet 1:805, 1971.

5.14 Muggia, F.M., and Uktmann, J.E. Reticulum cell sarcoma of the spinal epidural space. Ann. Int. Med. 75:477-478, 1971.

5.15 Ohnuma, T., Selawry, O.S., Holland, J.F., DeVita, V.T., Shedd, D.P., Hansen, H.H., and Muggia, F.M. Clinical study with bleomyci Tolerance to twice weekly dosage. Cancer. In press.

5.16 Roach, M.K., McIsaac, W.M., and Creaven, P.J. (eds) Biological Aspects of Alcohol. Austin, Texas: University of Texas Press, 19

5.17 Selawry, O.S., and Hansen, H.H. Lung Cancer in Cancer Medicine, Holland, J.F., and Frei, E., eds., Lea & Fibiger. In press.

5.18 Smith, J.C., Hansen, H.H., Howard, M.P., et al Plasma-zinc concentration in patients with bronchogenic cancer. Lancet 2: 1323, 1971.

5.19 Tucker, M., Creaven, P.J., and McIsaac, W.M. Preliminary observations on the metabolism of 1-^{14}C tyramine in man. J. Pharm. Pharmacol. In press.

5.20 Tucker, M., McIsaac, W.M., and Creaven, P.J. Effect of tranylcypromine sulphate on the metabolism of ^{14}C tyramine *in vivo* in the rat. J. Pharm. Pharmacol. In press.

SUMMARY

OFFICE OF THE CHIEF

BALTIMORE CANCER RESEARCH CENTER

The BCRC has had a highly productive year in its coordinated clinical-laboratory programs. Phase I-II clinical trials have been undertaken with Methyl CCNU, adriamycin, streptozotocin, guanazole and epipodophyllotoxin. Clinical pharmacologic studies with daunomycin, CCNU, guanazole, camptothecin and Methyl CCNU have demonstrated the degree of collaboration that can be obtained between the clinical and laboratory which evaluates drug metabolism, resistance and mechanism of action pari passu with therapeutic programs.

Phase II studies have demonstrated significant control of acute nonlymphocytic leukemia with daunomycin utilized according to schedules indicated as optimal by pharmacokinetic analysis of patient derived material. The elucidation of enzymatic pathways for the metabolism of daunomycin and the demonstration of daunomycin reductase and its effect on cellular uptake have added to our understanding of this important drug. Similar studies have been started with adriamycin. Combination drug therapy is being similarly evaluated with the hope of enhancing the superior effects of daunomycin by drugs with different modes of action on complementary schedules and attempting to abort occult CNS leukemic involvement. Reevaluation of old and new combination therapy regimens is constantly being undertaken.

Several new projects have been brought toward completion. A pilot randomized study of chemotherapy and x-ray therapy employed sequentially for the treatment of early (Stage I-II) Hodgkin's Disease has shown that local radiotherapy followed by multiple courses of combination drug therapy (MOPP) is more effective than the reversal sequence in preventing recurrence of the disease in non-irradiated areas. Exploratory laparatomy in early Hodgkin's Disease has continued to confirm the impression that clinical and radiologic criteria in assessing disease below the diaphragm is inaccurate in one-third of the cases. Combination therapy for advanced and refractory Stage III-B and IV Hodgkin's Disease is being explored and the diagnostic armamentarium now includes Gallium-67 scans in all patients with lymphoma.

The program of chemotherapy for CNS malignancy has been concerned with dose-scheduling and efficacy of the nitrosoureas integrated with pharmacologic evaluation of these drugs. A Phase II study has been completed and indicates that CCNU, like BCNU, produces complete or partial remission in about 40% of

patients with malignant brain tumor. A protocol evaluating high-dose epipodophyllotoxin weekly is being pursued. Methyl CCNU has been studied in a combined Phase I toxicity and pharmacology program. In regard to central nervous system leukemia, CSF clearance studies of drugs have been undertaken and the cyto-centrifuge has given the physician a tool which has been demonstrated to detect meningeal leukemia before other diagnostic tests are positive. Intrathecal chemotherapy is being examined from a pharmacologic and dose-relation point of view and increased laboratory effort in animal toxicology of intrathecal chemotherapy is being undertaken.

Biologic markers of occult neoplasm has advanced by the analysis of polyamines, carcinoembryonic antigen, alpha feto-protein and muramidase. The amines putrescine, spermine and spermidine, which appear in high concentrations in the urine of many patients, can now be detected with precision in concentrations heretofore unobtainable. The use of specific polynucleases and similar enzymatic approaches, as well as the gas liquid chromatograph, has provided the sensitivity necessary for these determinations. Specific RNAses have been prepared and refined for further analysis of oncogenic RNA.

Considerable improvement in infection control and prevention has been afforded through pioneer work and surveillance microbiologic culture techniques which define more precisely the patient at risk from infection and criteria for appropriate early institution of antibiotic therapy. These programs, along with an increasing use of the protected patient environment (Laminar Air Flow Units) have permitted a more confident and effective use of drugs to induce remission in leukemia. Unique microbiology culture programs, data retrieval systems and analysis systems have been broadened to facilitate patient care services in other select clinical areas of the NCI chemotherapy program. Viral and diagnostic studies and limited surveillance are being undertaken.

An especially designed on-floor patient care pharmacy service has provided control and supervision of drug utilization never before obtained. Trained pharmacists now take detailed drug and allergy histories, supervise unit-dose drug dispensing, prepare all intravenous adjuvant therapy, supply physicians with current drug-drug interaction information, and control drug use with a uniquely designed order form which is computer acceptable for detailed records in drug studies. The program of blood component therapy is a routine operation. Studies on platelet contamination and bacterial growth in units stored at room temperature have been undertaken. Use of the cell-separator, leukopacks and histocompatible donors for platelet-phoreses have

been developed. Utilized similar techniques, improved yield and stability of frozen and preserved normal and leukemic lymphocytes have been developed. Immunologic detection of host response to specific antigens and amplification of these responses by the use of synthetic polynucleotide complexes have played an important role in increasing the sensitivity of the host response. Immune complex disease utilizing immunofluorescent and electronmicroscopic studies has been shown in human kidneys of patients succumbing to neoplasm.

The Baltimore Cancer Research Center continues to vigorously participate in the Baltimore Medical and Scientific academic community. This is characterized by teaching, rounds and lectures which illustrate the scientific endeavors of this integral part of the National Cancer Institute.

SUMMARY REPORT

Clinical Branch, Baltimore Cancer Research Center, NCI

July 1, 1971 through June 30, 1972

This past year's efforts have produced significant advances in many project areas. Phase I studies have been carried out in Methyl CCNU, adriamycin, streptozotocin and guanazole in order to explore the toxicologic and therapeutic potential of these agents. Phase II studies have demonstrated significant control of acute nonlymphocytic leukemia with daunomycin utilized according to schedules indicated as optimal by pharmacokinetic analysis. Combination drug therapy is being similarly evaluated with the hope of enhancing the superior effects of daunomycin by drugs with different modes of action on complementary schedules and aborting occult central nervous system involvement with pyrimethamine. These investigations are being supplemented by re-evaluation of POMP and studies of maintenance cytoxan and guanazole. Diagnostic laparatomy in Hodgkin's Disease and other lymphomas has indicated that one-third of patients are inaccurately staged prior to exploration and, hence, their therapy would have been misguided. There are indications that the efficacy of chemotherapy following radiotherapy in Hodgkin's Disease is greater than the converse. Pilot studies in solid tumor chemotherapy, antibiotic regimens and hyperalimentation have been undertaken.

Phase II studies of CCNU in the treatment of malignant brain tumor have been completed showing a 40% remission in these patients. Concurrent neuropharmacologic evaluation of CCNU has been undertaken to evaluate oral absorption and tumor distribution characteristics. Epipodophyllotoxin (PTG) is currently being used in a Phase II study with some response being noted. Development of a clinical parenteral formulation of these aqueous insoluble nitrosoureas is nearing completion in the laboratory and significant enhancement of tissue concentrations demonstrated by this technique. Pharmacologic evaluation has been undertaken with adriamycin, daunomycin, Methyl CCNU and guanazole to better elucidate human pharmacodynamics and metabolism. Treatment of all patients has been vigorously monitored by a special patient care pharmacy service.

Protective environment (Laminar Air Flow) studies have been complemented by detailed microbiologic surveillance and diagnostic backup. This provides an understanding of the microbiologic environmental impact upon the patient and has indicated ways of reducing infective mortality. Specific studies have examined pseudomonas infection, colonization of the environment and improved culture taking and retrieval systems. Viral diagnostic

(Summary cont'd)

surveillance is now being undertaken. Biologic markers of malignant disease; muramidase, alpha fetoprotein, carcinoembryonic antigen and polyamines are being coordinated with the stage of disease and state of remission following chemotherapy.

To correlate this mass of data for analysis and teaching purposes, a pilot computer-organized problem-oriented medical system is being evaluated along with automated monitoring of drug therapy, the microbiologic reporting system and patient census programs.

The traditional role of the BCRC in the Baltimore academic community continues with participation in university and local hospital teaching programs and presentations at local, national and international meetings. A vigorous program for the forthcoming year includes evaluation of Phase I drugs, Phase II enhancement studies in leukemia, Hodgkin's Disease and brain tumors, and full-support research services including laminar air flow, isolation and blood component therapy.

Serial No.: NCI-6027
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Microbiological Services for Clinical and Research Studies for Cancer Patients at BCRC and at NIH, Clinical Center by Contracted Laboratory Facilities

Previous Serial Number: Same and NIH-6256. Also consider in conjunction with NCI-6219 and NCI-6259

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: I. J. Abramson, Ph.D., Robert C. Good, Ph.D., Gerald D. Vermeulen, M.D., Norman R. Friedman, B.S., Dolores M. Kenton, B.S., Arthur S. Levine, M.D., Cephus Patch, Ph.D., Janet Hauser, B.S.

Cooperating Units: Medical Service, NCI, BCRC. Leukemia Service, National Cancer Institute, N.I.H., Bethesda, Maryland, Hazleton Laboratories, Vienna, Virginia and Viral Diagnostics Laboratory, Georgetown University Hospital, Georgetown, University, Washington, D.C.

Man Years (July 1, 1971 - June 30, 1972)

Total:	3-1/2
Professional:	3-1/2
Other:	0

Project Description:

Objectives:

1. To supply viral and bacterial diagnostic services of a high quality for the study of actual and potential infection of cancer patients and of a versatility necessary to support cancer chemotherapy research programs.

2. To carry out environmental studies as well as ecological investigations of the patient flora, in support of individuals with cancer who will be placed in the microbiologically controlled Laminar Air Flow Rooms.

3. To investigate the behavior of viral agents in immunosuppressed patients whose bacterial flora has been suppressed by oral, non-absorbable antibiotics.

Methods Employed:

Samples for bacterial, viral and environmental studies are collected in transport media and/or on primary culture media. They are transported daily by chauffeur driven government vehicle to the contracted bacteriological or viral diagnostic laboratories. Daily review of presumptive results sent by teletype, frequent telephone conversations, site visits, and constant attention to computer generated final reports by the project officer ensures high quality information. Both contracted laboratories are inspected by the Center for Disease Control, Atlanta, Georgia and approved as well. The culture data are used both for patient management and as a basis for various other projects. In addition, this information is being recorded on computer mass storage discs and retrieved for research purposes.

Major Findings:

An average of 1,364 samples per month were processed by the contracting laboratory (Hazleton Laboratories) for recovery of bacteria during 1971. Approximately 775 to these were from the Medical Service, NCI, BCRC and 589 from the Leukemia Service, NCI, NIH. The latter includes both environmental and patient surveillance cultures. Numbers of samples for any one month vary widely, i.e., those from BCRC varying from 536 in Oct., to 994 in December.

Of 485 samples for viral isolation studied during a seven month period, 18% of the diagnostic samples and 3% of the surveillance samples yielded viral isolates. These included Enteroviruses from leukemia patients with aseptic meningitis, Herpes simplex from skin and mucous membrane lesions, parainfluenza virus from a patient with acute non-lymphocytic leukemia (ANLL) and Echo virus 11 was isolated from the pericardial fluid of another ANLL who died. During the same period 708 separate serologic determinations were performed on 50 patient sera. A significant rise in antibody titer was demonstrated to Herpes simplex,Coxsackie B, respiratory syncitial, cytomegalo, parainfluenza 1,and mumps viruses.

This program has proven that outside contract facilities can provide accurate and current microbiologic laboratory information for daily patient care. The laboratories' use of computerized reporting, storage and retreival of the information generated has facilitated the reporting of the data and its use in clinical care and research. Continuous collaboration, monitoring of information and exchange of cultures have verified the high degree of accuracy of reported results. The responsiveness of the Contractors requests for such things as serotyping Klebsiella spp., minimum inhibitory concentrations performed by Microtiter on designated strains of bacteria to monitor development of antibiotic resistance, stocking of all Pseudomonas, Staphylococcus aureus, etc., strains for future studies has provided the basis for a number of valuable retrospective investigations which would not have otherwise been possible.

Significance to Bio-medical Research:

The successful operation of contracted bacteriologic and viral services for use in direct patient care has been demonstrated. It provides the clinician with immediate information for use in patient care and a basis for better understanding of infection in cancer patients, as well as the sources of endogenous microorganisms. The data generated by the program also provided the base for a number of research investigations. (Project Nos. 6217, 6223, 6224, 6253, 6264, 6266, 6267.

Proposed Course:

To continue the program along the same lines.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6039
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Computerized Microbiology Reporting and Patient Information System for use by a Distantly Located Contracted Laboratory Facility

Previous Serial Numbers: NCI-6220, 6254 and same

Principal Investigator: Gerald D. Vermeulen, M.D.

Other Investigators: Viola Mae Young, Ph.D., Robert Esterhay, M.D., Richard K.C. Hsieh, Dr. PH., Richard Foard, John Gerster, and William L. Robinson, W.H. Greene,M.D.

Cooperating Units: Medical Service, NCI, BCRC; Health Services Research, U.S. Public Health Service Hospital, Balto., Md. 21211

Man Years (July 1, 1971 - June 30, 1972)

Total:	3
Professional:	1-1/2
Other:	1-1/2

Project Description:

Objectives:

1. To provide a means of permanently recording B.C.R.C. patient data for use by Hospital clinicians and contract laboratory personnel

2. To establish a practical method of recording and reporting preliminary clinical microbiologic laboratory data by computer, for use by a distantly located laboratory facility.

3. To provide a means of automating various clerical tasks made necessary by the use of contracted laboratory facilities and other clerical functions necessary in the operation of a clinical microbiology laboratory.

Methods Employed:

Information is entered from both the hospital and distantly located microbiology laboratory facilities to common data files which are stored on Tymshare timesharing computer system. The data may then be accessed from the hospital and from the distant laboratory or may be transferred to magnetic tape at the hospital computer center for permanent storage of

batch processing. Final microbiology data is batch processed at the hospital computer center and permanently stored on mass storage disc which is used as the data base for data retrieval.

Major Findings:

A patient census program (see Project Report NCI-6220) has been in operation since August, 1971 which records vital statistics, room and beds occupied and therapy information in permanent time-sharing data files. A daily census report is produced, listing the inpatients, outpatient visits, diagnosis, attending physician, etc. This data may also be accessed from the distantly located contracted laboratories. The data recorded in these data files is also used by other programs to detect errors in hospital numbers, names, etc., as well as for accounting and other data summaries.

The microbiology preliminary reporting system is now under development. This will allow microbiologic specimen data to be entered from the hospital and from the distantly located laboratory facilities. The data in these files will be accessed by programs used by both the laboratory and the hospital to enter identifying information and results of laboratory studies and to generate preliminary reports for the clinicians. After the laboratory has completed the necessary studies, the final information will be transmitted to the hospital computer center for batch processing of the data.

The microbiology final report generating, data storage and retrieval system has been in successful operation since 1969, as previously reported, (see NCI-6039). Retrieval programs provide for the retrieval of data by multiple parameters and is used in patient care as well as for microbiology research purposes. The data bank contains all the data generated since the institution of the first microbiology contract in July, 1968, and contains in excess of 60,000 data records.

Significance to Bio-medical Research:

The development and operation of the census program provides the distantly located contracted laboratory facilities with the means of obtaining current patient information needed for optimal processing of the clinical specimens. The information which is being recorded provides data for hospital census reports and for the study of the transmission of specific microorganisms within the hospital. The data also provides the means of developing accurate accounting and administrative reports.

The microbiology preliminary reporting system is now under development. This will provide the means of entering information to time-sharing files from the hospital as well as from the distantly located laboratories which will be used by the hospital for the production of current preliminary microbiology reports and data retrieval. Final data will be transferred to magnetic tape for batch processing and storage at the hospital Computer Center.

This automated method of reporting of preliminary microbiology information will eliminate an estimated 50% or more of the time-consuming clerical work which is necessary to record and maintain preliminary laboratory results and other data essential to the operation of a clinical microbiology laboratory. The preliminary reporting system will be interfaced with the final reporting system and will expedite the processing of the final data. The successful development of this type of system will greatly facilitate the reporting of preliminary microbiology information, now a major obstacle in the establishment of contracts for routine clinical microbiology laboratory services and will be one of the first of its type in operation.

The final microbiology data reporting, storage, and retrieval system has been previously described (see Project No. NCI-6039). The data bank is now disc based and has been revised to decrease the time necessary for retrieval.

Proposed Course:

Additional programs will be written to utilize the data recorded by the operation of the census program to provide accounting information and various other purposes. Modification will also be made to simplify its operation, etc., as needed.

The microbiology preliminary reporting program is soon to be put into operation and changes will be made as indicated. Future development will be directed toward providing automated methods of record keeping, data summarization, etc.

The microbilogy final report generating, data storage and retrieval system will continue to be expanded and new programs will be written as the need dictates.

Honors and Awards:

Panel discussant for a workshop on"Practical Techniques in the Computerization of Clinical Microbiology"sponsored by the Eastern Pennsylvania Chapter of the American Society for Microbiology, April, 1972, Viola Mae Young, Ph.D.

Presentation No. 1 (below) chosen for press release at the American Society of Microbiology Meeting, Boston, Mass., April 26, 1970.

Presentations:

1. American Society of Microbiologists, Boston, Mass., April 26, 1970, "Use of Computers in Reporting Microbiologic Results By Contracting Laboratories,": G. D. Vermeulen, V.M. Young, S.V. Schwab, R.K.S. Hsieh.

2. USPHS Clinical Society, Washington, D.C., April 2, 1970, "Use of Computer Facilities in the Monitoring of Microbiologic Flora of Cancer Patients,"Gerald Vermeulen, M.D.; Viola M. Young, Ph.D.; Dolores M. Kenton,

B.S.,; Marcia Moody, M.S.; Stephen Schwab, B.A.

3. Clinical Microbiology Section, Eastern Pennsylvania Chapter of the American Society for Microbiology, Philadelphia, Pa., January 18, 1971, "The Use of Computers by Clinical Microbiologists," Viola Mae Young, Ph.D.

4. American Society of Microbiologists, Minneapolis, Minn., May 3, 1971, Microbiologic Data Retrieval: Systems and Information Utilization," Gerald Vermeulen, V.M. Young, and R. H. Hsieh.

Publications:

A Computerized system for Clinical Microbiology, Gerald D. Vermeulen, Stephen V. Schwab, V. M. Young, and Richard K. Hsieh, Published in American Journal of Clinical Pathology, Vol. 57,No. 3, March, 1972.

Computer Program for Monitoring Antibiotic Susceptibilities and Bacterial Infections in a General Hospital, J.R. Reed, V.E. Kilpe, M.D., G.D. Vermeulen, M.D., L.S. Reich and V.M. Young, Antimicrobial Agents and Chemotherapy - 1970, American Society for Microbiology (1971).

Serial No. : NCI-6219
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies Directed Toward Elucidation of Cancer Patient Microbial Flora, Colonization by Specific Bacterial Genera and Infection

I. The Detection of Pneumocystis carinii Infection in Cancer Patients

II. An Investigation of Klebsiella spp. Isolated from Cancer Patients, their Serotype and Antibiograms

III. A Study of the Incidence and Types of Gram Negative Rods and other Pathogenic Microorganisms in the Oral Cavity of B.C.R.C. Patients in Relationship to Dentition

IV. Broad Spectrum Bacterial Antibody Evaluation of Cancer Patient Sera

V. A Proposed New Genus of the Family Enterobacteriaceae

VI. Incidence of Antibodies against Heterogenetic Enterobacterial Antigen in Typhoid Vaccinated Subjects

Previous Serial Number: Same and Project Nos.: 6218, 6255, 6036, 6031, 6035

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Marcia R. Moody, M.S., Robert C. Good, Ph.D., Gerald D. Vermeulen, M.D., Dolores M. Kenton, B.S., Don J. Brenner, Ph.D.

Cooperating Units: Medical Service, NCI, BCRC, Hazleton Laboratories, 9200 Leesburg Turnpike, Vienna, Virginia, Division of Biochemistry, WRAIR, Washington, D.C.

Man Years (July 1, 1971 - June 30, 1972)

Total:	1-1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

The objectives of these studies are as follows:

1. To develop a serological test or other procedure to detect infection with Pneumocystis carinii, a not uncommon finding in immunosuppressed cancer patients. The only procedure presently recognized to give a reliable diagnosis is lung biopsy, a potentially hazardous procedure for cancer patients.

2. To serologically identify Klebsiella strains to determine prevalence of a particular type and possible endemicity of such types within the hospital environment.

3. To determine the species and incidence of gram negative rods and other pathogens in the oral cavity of cancer patients, to relate them to dentition of the patient and/or eventual infection.

4. To determine "normal" antibody levels in cancer patients as they enter the hospital to a variety of antigens prepared from microorganisms which most frequently cause infection, to monitor changes in antibody titer during their hospital stay and as infection occurs.

5. To determine a possible heterogenetic antibody response (with its implied protection against infection with a number of the members of the Tribe(Enterobacteriaceae) to vaccination with Salmonella A,B, and D vaccine. The common antigen of this group of organisms might serve as a stimulus for such protective antibodies.

6. To specifically identify bacteria which colonize cancer patients and which belong to no hitherto described genera. It is important to speciate such strains for future recognition and to examine possible differences in antibiotic susceptibilities from other related species.

Methods Employed:

Pneumocystis carinii antigens are to be used in complement fixation, gel diffusion and perhaps other serological techniques to attempt to achieve early detection of infection.

Biochemical reactions were used to classify Klebsiella species and Difco antisera utilized to perform the capsular swelling test.

All gingival, mouth and throat samples are coded when taken to indicate the

state of the patient's dentition. Culture results are retrieved by computer programs and reviewed for significance of microorganisms, state of dentition and infection relationships.

Standard Microtiter hemagglutination and electrophoretic techniques were employed for determining serum antibody levels.

Standard biochemical and serological procedures, minimal medium techniques, oxidation-fermentation media and electron microscopy were used to study new and/or unusual microorganisms recovered from cancer patients. G-C ratios were determined for the Genus Levinea and other enteric bacteria.

Standard hemagglutination and bacterial agglutination procedures were used to study heterogenetic antibody response to Salmonella vaccines.

Major Findings:

It took a longer period of immunosuppression of rats then was reported by previous investigators to produce heavy infestation of the rats with Pneumocystis carinii. Antigen containing large number of Pneumocystis has been successfully prepared and gel diffusion studies with this antigen and sera procured from the rats at various stages of their infection are being carried out.

Klebsiella spp. were the most widely spread of any organism by colonized patients. Most such Klebsiella were the same serotype as carried by the patient. Sinks and sink drains have been found to harbor other serotypes. Results indicate that infections have been caused by a number of different serotypes.

Gram negative rods which comprise part of the normal intestinal flora and which frequently cause infection in immunosuppressed cancer patients occur with unusual frequency in their oral cavity. This finding does not appear to be related to dentition.

Patient sera are collected on a regular basis for future studies on their antibody levels against antigens which are being prepared from a number of the strains that have colonized or caused infection in the patients.

In collaboration with investigators at walter Reed Army Institute for Research, relatedness was demonstrated between the two species of the proposed new genus Levinea, described in the prior Project Report, and other members of the Tribe Enterobacteriaceae. These organisms differ from all previously described strains, but the species L. malonatica appears to be related to the genus Citrobacter diversus, since reported by Ewing. L. amalonatica appears to be closely related to an H_2S negative, indole positive Citrobacter fruendii, yet the two "Levinea species share identity, genetically and serologically.

Another new hitherto undescribed microorganism which colonized a cancer

patient for over two months has been intensively studied. It is a peritrichously flagellated gram negative rod which ferments only pentoses.

Other lots of Salmonella vaccine were tested for the presence of heterogenetic antigen and none was demonstrated.

Significance to Bio-medical Research:

All of these studies have helped to define the species of microorganisms which colonize patients, the location of patient sites which are colonized, and enables a better understanding of infection in the patient as well as the patient's response to that infection.

Proposed Course:

It is proposed to continue these studies along the lines indicated except for Project No. 6035 which has been completed.

Honors and Awards: None

Presentations:

Colonization of a Cancer Patient by an Unusual Pentose-Fermenting Gram Negative Bacterium, presented by Marcia Moody, M.S., at the 72nd Annual Meeting, American Society for Microbiology in Philadelphia, Pa., April, 1972.

Publications: None

Serial No.: NCI-6223
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Pseudomonas aeruginosa in a Cancer Research Center

I Distribution of Immunotypes from Human and Invironmental Sources

II Comparison of Immunotypes from Cancer and non-Cancer Patients

III Hemagglutinin Levels to Pseudomonas aeruginosa in Cancer Patients

IV Incidence of Pseudomonas species other than P. aeruginosa in Cancer Patients

V Relationship of Pseudomonas aeruginosa Immunotypes to Antibiotic Sensitivity Patterns

VI The Effect of Various Surfactant Chemical Agents on the Serum Sensitivity of Pseudomonas aeruginosa and the Influence of such Phenotypic Conversion on Host Defense Mechanisms

VII Phagocytosis of Pseudomonas aeruginosa by Immune and Non-immune Guinea Pig Macrophages

Previous Serial Number: Same and Project Nos.: 6222, 6224, 6252, 6253, 6251, 6221. Also consider in conjunction with NCI 6030, 6264

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D., Stephen C. Schimpff, M.D., W.H. Greene, M.D.,Dolores M. Kenton,B.S., Myron Fisher,Ph.D.

Cooperating Units: Parke Davis and Co., Detroit, Michigan

Man Years (July 1, 1971 - June 30, 1972)

Total:	3-1/2
Professional:	1-1/2
Other:	2

Project Description:

Objectives:

Pseudomonas aeruginosa is the most frequent cause of infectious death in patients receiving chemotherapy for acute myelocytic leukemia and a frequent cause of infection in other immunosuppressed cancer patients. The main objectives of this project are to determine the distribution of Pseudomonas immunotypes from environmental sources; to compare them with those recovered from cancer and non-cancer patients; to determine the relationship of the immunotypes to antibiotic susceptibility patterns; to identify certain aspects of this organism's pathogenic effect and the patient's response. Also other Pseudomonas spp. which colonize the patients are to be evaluated and their susceptibilities determined.

Methods Employed:

All Pseudomonas strains recovered from the patients and their environment were speciated and all P. aeruginosa strains were immunotyped using antisera supplied by M. Fisher of Parke, Davis and Co. Hemagglutinin levels of patient antibodies were determined by the microtiter technique. Antimicrobial susceptibility tests were performed by the Bauer-Kirby-Sherris-Turck techniques. Pseudomonas strains other than P. aeruginosa recovered from cancer patients were speciated by a schemata derived from the systems of Stanier (minimal medium), Hugh (oxidative-fermentative (O-F reactions) and Pickett (O-F reactions utilizing buffered media). Macrophages from guinea-pigs were incubated with an immunizing serotype of P. aeruginosa and the phagocytic endex determined.

Major Findings:

This study has revealed that P. aeruginosa types 1,2,6, and 7 are prevalent among cancer patients. Type 1 is a common serotype which is prone to transitory colonization of patients and causes severe infection less often than types 6 or 7 that tend to become established as resident flora. All serotypes of P. aeruginosa were recovered frequently from the oropharyngeal, respiratory and gastrointestinal areas. However, types 3 and 6 had a statistically significant affinity for the mouth and respiratory tract. Types 2,4, and 7 were isolated from the blood of approximately one-fourth of the patients colonized with these strains. Types 2 and 4 have both been recovered from water sources followed by an increased recovery from patients. The schemata utilized for species differentiation has provided a means for proving that Pseudomonas spp. other than P. aeruginosa are often present in water supplies and that they colonize patients and become resident flora. It has afforded the opportunity to us to observe that some species, particularly P. multivorans,have developed resistance to all currently utilized antibiotics, including polymixin B and colymycin and that this species has colonized increasing number of patients. It was also found that susceptibility tests of these pseudomonads were more stable than those of P. aeruginosa and could be utilized for presumptive identification of the species.

Systems have been developed for checking the phagocytic indices of guinea pigs for P. aeruginosa. Animals have been immunized for production of the immune phagocytes and comparison of the immune and non-immune systems are in progress.

Significance to Bio-medical Research:

These studies have led to a greater understanding of the dynamics of interaction between Pseudomonas aeruginosa in the environment and in the patient. They have made it possible to define related flora and to prepare for a potentially dangerous ecologic situation wherein resistant organisms have colonized a number of susceptible patients. They have also allowed us to predict the likelihood of severe infection in certain patient groups and to better understand the mechanism of the disease produced.

Proposed Course:

1. Project No. 6223 is completed.
2. Project No. 6222. The work is completed and a publication is in preparation.
3. Project No. 6224. More patient antisera have been collected, the automated microtiter system for determination of hemagglutins levels developed and the serologic studies will be continued.
4. Project No. 6253 will be continued using the schemata which has been developed.
5. Project No. 6251 has been completed, but studies of Pseudomonas susceptibility patterns will continue to be monitored for the possible emergence of resistant strains.
6. Project 6221 will not be continued as other investigators have shown in the interim that surfactant chemicals which alter lipid in the wall of Pseudomonas aeruginosa does make the organism more serum sensitive and enhance the bactericidal effect. These chemicals also make P. aeruginosa more susceptible to antibiotics.
7. Project No. 6252 will continue along the lines indicated.

Honors and Awards: None

Presentations:

Antibiotic Susceptibility of Pseudomonads other than P. aeruginosa Recovered from Cancer Patients, presented by Marcia Moody, M.S., at the 11th Interscience Conference on Antimicrobial Agents and Chemotherapy in Atlantic City, October, 1971.

Publications:

Pseudomonas aeruginosa in a Center for Cancer Research. I. Distribution of Intraspecies Types from Human and Environmental Sources, Journal of Infectious Diseases, February 1972, 95-100, Moody, M.R., Young, V.M., Kenton, D.M., and Vermeulen, G.D.

Empiric Therapy with Carbenicillin and Gentamicin for Febrile Patients with Cancer and Granulocytopenia. New Engl. J. Med. 284: 3-7,1971. Schimpff, S.C., Satterlee, W., Young, V.M., and Serpick, A. (also listed under NCI-6266.

Relationship of Colonization with Pseudomonas aeruginosa to Development of Pseudomonas Bacteremia in Cancer Patients. Antimicrobial Agents and Chemotherapy 1970-1971, pp. 240-244, Schimpff, S.C., Moody, M.R., and Young, V.M. (also listed under NCI-6264).

Serial No. NCI-6254
1. BCRC
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Research Activities Directed Toward Improvement of Microbial Culture Techniques

I. A Comparison of Buffered Glycerol Saline, Todd-Hewitt Broth and Unpreserved Fecal Material as the Means of Sample Collection to Determine Fecal Microbial Flora

II. A Study of Relative Effectiveness of Different Types of Blood Culture Media and Laboratory Techniques for the Isolation of Specific Types of Microorganisms

III. A Study of the Efficacy of Selective Media for the Recovery of Pseudomonas spp.

Previous Serial Nos.: 6254 and 6257

Principal Investigator: Gerald D. Vermeulen, M.D.

Other Investigators: Viola Mae Young, Ph.D., Robert C. Good, Ph.D.

Cooperating Units: Hazleton Laboratories, 9200 Leesburg Turnpike, Vienna, Virginia

Man Years (July 1, 1971 - June 30, 1972)

Total:	1/3
Professional:	3/12
Other:	1/12

Project Description:

Objectives:

Most bacteremias in leukemia patients are endogenous in origin and are chiefly caused by Pseudomonas aeruginosa. Early detection of these organisms prior to and during development of bacteremia are essential to adequate patient care. These studies were undertaken to find the optimal methods for sampling and preserving the total aerobic fecal flora of cancer patients, for growth of organisms from blood of patients with bacteremias and for cultivation of P. aeruginosa from samples heavily contaminated with other microorganisms.

Methods Employed:

Todd-Hewitt Broth, buffered glycerol saline and unpreserved fecal material were compared for their ability to retain viability of fecal organisms during transport to the laboratory. Tryptic soy broth (Difco), brain heart infusion broth with 30% sucrose and 0.1% agar (Difco), thiol (Difco), brain heart infusion broth with cysteine under nitrogen and thioglycollate broth are under comparison for their ability to support growth of organisms from blood. Cetrimide agar (Pseudosel Agar, BBL) and Pseudomonas Isolation Agar (Difco) were evaluated by comparing them with routine culture media for the recovery of Pseudomonas aeruginosa.

Major Findings:

Over a thousand fecal and/or perirectal cultures were compared for recovery rates from the various media. There was no significant difference in recovery rates for Enterobacter spp. and Pseudomonas spp. Todd-Hewitt broth was significantly superior to buffered glycerol saline for recovery of other organisms. More organisms were recovered from perirectal swabs of patients on oral non-absorbable antibiotics than from rectal swabs or stool.

Tryptic soy broth and brain heart infusion broth give similar recovery rates for organisms from blood. Evidence continues that multiple types of media are necessary for optimal results.

The use of cetrimide agar for the isolation of P. aeruginosa was not significantly better than routine media, however, Pseudomonas isolation agar was significantly superior to routine media in the recovery of non-aeruginosa strains.

Significance to Bio-medical Research:

Optimal bacterial culture techniques are essential to understanding of patient status and care and provide an integral part of the patient care.

Proposed Course:

The proposed course is to proceed with this type of investigation as new cultural techniques become available to provide a continuing method of evaluation and of obtaining optimal results.

Honors and Awards: None

Presentations:

A Comparison of Recovery Rates of Gram Negative Rods Cultured from Rectal Swabs Held in Buffered Glycerol Saline and Todd-Hewitt Broth, presented by Gerald Vermeulen, M.D., at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania, 1972.

Publications: None

Serial No. NCI-6217
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Prevention of Infections in Patients with Cancer Including Evaluation of Protected Environments and Surveillance Cultures

Previous Serial Number: 6265, 6217, 6029

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: Viola Mae Young, Ph.D., William H. Greene, M.D., Clarence L. Fortner, M.S., Lois Jepsen, R.N., Peter H. Wiernik, M.D., Michael D. Walker, M.D., Dennis Duda, M.Ed., Nancy Cusack, I.J. Abramson, Ph.D., Norman Friedman, B.S., Dolores Kenton, B.S., Marcia Moody, M.S.

Cooperating Units: Research Microbiology Section, BCRC; Center for Disease Control, Atlanta, Georgia; Myron Fisher, Ph.D., Parke Davis and Company, Detroit, Michigan

Man Years

Total:	5 1/2
Professional:	3 1/2
Other:	2

Project Description:

Objectives:

Infection is the most common cause of death in patients with acute leukemia and is a major cause of death in all other types of cancer, especially those receiving cancer chemotherapy. At this Center, the most common cause of infectious death is Pseudomonas aeruginosa. As noted in another project (6264), approximately half of the microbiologically documented infections in patients with acute leukemia were due to organisms which had been acquired following admission to the hospital. Again, the most prevalent cause of infection was Pseudomonas aeruginosa. Therefore, various avenues have been pursued in an attempt to reduce the acquisition of hospital organisms and to prevent infection by the patient's endogenous flora.

A) Protected Environment with the Use of Laminar Air Flow Rooms and/or Oral, Nonabsorbable Antibiotics - All adult, non-infected patients with acute nonlymphocytic leukemia are randomized into one of the following three groups: 1) laminar air flow unit plus ingestion of oral, nonabsorbable antibiotics,

Objectives (cont'd):

2) ward care with oral, nonabsorbable antibiotics, or 3) ward care without antibiotics. Patients in the three groups are all treated identically with respect to nursing care, physician care, and supportive care. Patients in the laminar air flow room are treated under sterile conditions, using sterile supplies, and a cooked food diet. All infections are carefully observed and recorded.

B) Clinical Trials of Polyvalent Pseudomonas Hyperimmune Globulin - Because of the high incidence of Pseudomonas aeruginosa infection and because vaccination against the seven common immunotypes of Pseudomonas aeruginosa has been successful in reducing the incidence of sepsis in burn patients, a trial of Pseudomonas aeruginosa hyperimmune globulin has been studied in a randomized, controlled manner in patients with acute nonlymphocytic leukemia. Patients randomized to receive the polyvalent hyperimmune globulin received 20 cc every two weeks while their total white count was below $1000/mm^3$; controls received 20 cc of commercial gamma globulin under the same regimen. Serum samples are collected to determine titer rises. Twice weekly cultures of stool and gingiva are obtained. Patients are examined regularly for evidence of infection.

C) Studies to Determine the Routes of Hospital Acquisition of Potential Pathogens - Cultures of multiple sites in the hospital environment are taken by the Research Microbiology Laboratory, with emphasis on water sources such as ice machines, respirators, sinks, and sink drains. Cultures of physicians', nurses', and technicians' hands are taken at intervals. Careful observations of handwashing techniques, sterilization techniques, food preparation, and patient care are in progress. All Pseudomonas strains are speciated and serotyped (Fisher method), Klebsiella spp. are serotyped, Staphylococcus aureus are phage typed, and some Proteus spp. are proticine typed. This data is correlated together in an attempt to determine how and from where a cancer patient acquires potential pathogens from the hospital environment.

Major Findings:

A) Laminar Air Flow Rooms - As of April 1, 1972, there have been 47 patients with ANLL admitted of which 35 were eligible for randomization; 13 were treated in the laminar flow room, ten on the ward with antibiotics, and 12 on the ward without antibiotics. All patients had equal degrees of granulocytopenia; all were treated until complete remission or death. Although it is too early in the study to show statistical significance, there is a tendency toward fewer infections in the laminar air flow patients and the ward patients who are receiving antibiotics when compared to the ward patients who are not receiving antibiotics. It also would appear that patients in the laminar air flow room or on the ward receiving antibiotics are less likely to die of or with infection than the patient in the ward without antibiotics group. Furthermore, there seems to be a reduction in the incidence of pneumonia in the laminar air flow group and the ward plus anti-

Significance to Cancer Research (cont'd):

tance. Besides for the obvious advantage of reducing infection, a prolongation of life will allow for further and more effective cancer chemotherapy. For patients with leukemia, this may mean that more patients will achieve a complete remission which is now the only known way of significantly prolonging useful, out-of-hospital life.

Honors and Awards: None.

Publications:

Schimpff, S., Moody, M., Young, V.M.: Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. Antimicrobial Agents & Chemotherapy 10:240-244, 1970.

Presentations:

I Dr. Schimpff

1. Laminar air flow units for patients with leukemia, Infectious Disease Seminar, Section of Infectious Diseases, University of Maryland School of Medicine, December 7, 1971.

2. Reverse isolation on a cancer service, Grand Rounds, D.C. General Hospital, Georgetown Medical Service, March 25, 1971.

3. Protected environment program at the BCRC. Second Annual Contractors Meeting, Annapolis Hilton Hotel, October, 1971.

4. Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy. October, 1970. Chicago, Illinois.

II Dr. Greene

1. A controlled clinical trial of unidirectional air flow reverse isolation and oral antibiotic intestinal flora suppression in decreasing infection in the leukemic patient. Eastern Section of the American Federation for Clinical Research, Philadelphia, Pennsylvania, December, 1971.

III Dr. Young

1. A study of microorganisms recovered from a laminar air flow room during use as a protective environment for acute leukemia patients undergoing chemotherapy, Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania,

2. The behavior of endogenous and exogenous gram negative rod flora of leukemia patients in an open ward and laminar air flow environments, 11th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, New Jersey,

Major Findings (cont'd):

biotics group when compared to the ward alone group; likewise there is a reduction in ano-rectal infections and urinary tract infections. No patient taking the oral, nonabsorbable antibiotics has had the development of a resistant organism. However, one patient while taking the oral antibiotic combination acquired a gentamicin-resistant Pseudomonas aeruginosa from another patient. Five patients, assigned to take the oral, nonabsorbable antibiotics, discontinued these drugs of their own accord, and four of these five patients went on to develop infection which in each case, was a Pseudomonas aeruginosa bacteremia which led to their demise. Thirteen patients who had taken the oral, nonabsorbable antibiotics had now reached remission status; of these 13 patients, four had developed subsequent infection while in complete remission and off the antibiotics. However, all four patients survived the infection, and three of the four patients developed this subsequent infection in association with consolidation therapy of their leukemia.

Although the preliminary data is encouraging, further patient trials will be necessary in order to firmly establish whether or not laminar air flow rooms and/or oral, nonabsorbable antibiotics have a place in the therapy of patients with acute nonlymphocytic leukemia.

B) Pseudomonas Hyperimmune Globulin - Twenty patients have been entered into each arm of the protocol. A total of three Pseudomonas aeruginosa infections have been encountered. These numbers are too small to make meaningful comparisons. No significant side reactions have been encountered. No appreciable titer rises have occurred. The Parke Davis and Company has discontinued manufacture of the hyperimmune globulin and so the study has, for the present, been discontinued.

C) Studies to Determine the Routes of Hospital Acquisition of Potential Pathogens - Hand cultures of the hospital personnel over the past two years have shown that 43 of 126 (34%) were contaminated with either Staphylococcus aureus or gram negative bacilli. Surveillance culturing of the LAF rooms, wards, intensive care units, etc., and serologic identification of microorganisms as in (C) OBJECTIVES when utilized together with the Census Program (see Project No.) to follow the location of the patients within the ward or rooms and served to monitor spread of bacterial pathogens. This information is then used to prevent the build-up of organisms that often precedes an epidemic and to point out sources of spread as when procedures were inadequate or when certain patients were shedders of pathogenic bacteria.

Proposed Course: Continue each of the three investigations.

Significance to Cancer Research:

A significant reduction in the incidence of infection and/or the incidence of infectious death in this patient population would be of considerable impor-

Serial No.: NCI-6228
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Preliminary Clinical and Pharmacological Evaluation of Camptothecin Sodium NSC 100,880

Previous Serial Number: Same

Principal Investigator: J.B. Block, M.D.

Other Investigators: A.M. Guarino, M.D.
V.T. Oliverio, M.D.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To determine the appropriate dosage schedule for this new anti-tumor agent and to carefully observe the toxicities it produces. To determine the anti-tumor spectrum of this drug and compile accurate data regarding it palliative effects. To determine the drug's pharmacological properties.

Methods Employed:

Patients are given camptothecin sodium intravenously at varying dosage levels over varying periods of time. Plasma, urine, other body fluids and tissues are collected at various times and analyzed for camptothecin levels, using a fluorometric spectrophotometric method highly specific for camptothecin.

Major Findings:

1. A dose of 7.5 mg/Kg given I.V. push over 5 to 10 minutes every 2 to 3 weeks is well tolerated. Higher doses produce too great a granulocytopenia and thrombocytopenia to be useful, while lower doses have not produced appreciable tumor response. Bi-weekly doses of 25 and 37.5 mg/M^2 have not produced responses; 50 mg/M^2 may produce a response but toxicity is

Major Findings: (Cont'd)

appreciable.

2. Camptothecin is neither as active against gastrointestinal cancer nor as non-toxic as was originally thought -- the response rate observed here now being 4 responses in 17 patients. Alopecia, nausea, vomiting and hemorrhagic cystitis have occurred in many of the patients treated, in addition to marrow depression.

3. Camptothecin has an initial rapid plasma disappearance followed by a slower rate. No drug is detectable in urine or plasma beyond the sixth day in patients with normal renal function. Up to 40% of unmetabolized drug can be found in the first 48-hour urine collection. No drug has yet been detected in body tissues.

Significance to Cancer Research:

Camptothecin appears to be a powerful mitotic inhibitor with some activity against gastrointestinal tract neoplasms, which are relatively unresponsive to chemotherapy. Its minor efficacy and great toxicity suggest that this drug will not be a useful tool.

Proposed Course:

Terminate the study.

Publications: None

Serial No.: NCI - 6230
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease.

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: John Sutherland, M.D., Irwin Zarembok, M.D.
Harold E. Ramsey, M.D.

Cooperating Units: Pathology and Tumor Surgery Departments, USPHS Hospital

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives:

Accurate determination of the extent of lymphomatous involvement with Hodgkin's Disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's Disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Major Findings:

Over 75 patients with Hodgkin's Disease have undergone laparotomy and splenectomy for staging. The preoperative assessment of splenic involvement was incorrect in 1/3 of the patients studied. Many spleens of normal size were found to be involved and some large spleens were free of disease. Some positive lymphangiograms could not be confirmed at surgery. Involvement of nodes in the splenic hilum, porta hepatis and mesentery was demonstrated in many patients with extensive abdominal disease. Hepatic involvement has been found despite normal liver function tests and scans. There has been no operative mortality and minimal morbidity. Tolerance to extended field

Major Findings: (Cont'd)

radiotherapy and combination chemotherapy may be improved as a result of splenectomy.

Proposed Course:

Continue investigation.

Honors and Awards: None.

Publications: Zarembok, I., Ramsey, H.E., Sutherland, J., and Serpick, A.A.: Laparotomy and Splenectomy in the Staging of Untreated Patients with Hodgkin's Disease, Radiology, 102:673-678, 1972.

Serial No.: NCI-6259
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Conversion from the Traditional or Source Oriented Medical Record (SOMR) to the Problem Oriented Medical Record (POMR) in Paper/Manual Form (M-POMR) and Computer Form (C-POMR)

Previous Serial Number: Same

Principal Investigator: Robert J. Esterhay, Jr., M.D.

Other Investigators: Peter H. Wiernik, M.D.
Michael D. Walker, M.D.
Viola M. Young, PhD
Sarah Chamberlain, R.N.
Clarence Fortner, Pharmacist

Cooperating Units: PROMIS Laboratory
Department of Medicine
University of Vermont
Burlington, Vermont 05401
Telephone: (802) 656-3946

Cooperating Investigators: Lawrence L. Weed, M.D.
Laura B. Weed, M.D.
George E. Nelson, M.D.
Jan R. Schultz, Research Associate
Stephen V. Cantrill, Research Programmer
Keith G. Morgan, Research Programmer

Man Years:

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

To convert the traditional or source oriented medical records at BCRC to the Problem Oriented Medical Record, in paper/manual form (M-POMR) and concurrently to computer form (C-POMR) utilizing the PROMIS Laboratory computer system via telephone lines with a touch sensitive cathode ray tube

Objectives: (Cont'd)

terminal that allows structured input by a directly interfaced medical user (in particular the physician, nurse and clinical pharmacist).

Methods Employed:

The system whether in paper/manual (M-POMR) or computer form (C-POMR) requires medical data to be collected, filed and identified with respect to a given problem and not to the source of the data as in the "traditional" record.

Major Findings:

The M-POMR is now totally operational for all services, at BCRC, in-patient and out-patient. It has improved the audit of patient care and clinical research at BCRC. The C-POMR has just become operational and will be extensively tested in the next fiscal year.

Significant to Cancer Research:

The system encourages the physician, nurse and clinical pharmacist to use sound logic in his thoughts about patients -- not only the cancer problem, but all problems -- medical, social, psychiatric and demographic. The system allows the physician to communicate to all others who have immediate care of the patient. Inherent in the system is self-continuing education of the physician and all who assist in the care of the patient. An attending physician on the cancer service using the system can check a patient's problems and audit the clinical associate's ability to collect a data base to define the problems, to develop initial plans and to follow-up on patient's problems properly. Clinical investigation requires excellent records. The system makes retrospective review of data easier since data is entered and therefore in sequence by problem. With the C-POMR clinical research information (for example,flow sheets) is a by-product of patient care as opposed to an additional recording task. All of the above reasons will enable the physician to improve the care of his patients, structure his learning and communications to others about his patients, and collect more accurate clinical research data since a patient's problems can be retrieved and looked at in context to each other and in proper time relationships.

Proposed Course:

Continued study.

1. The BCRC staff will develop the necessary displays for the research treatment protocols for acute leukemias, lymphomas, brain tumors and selected solid tumors which will be integrated with the displays already on file in Burlington, Vermont as part of the PROMIS system (currently over 16,000 displays have been developed by the PROMIS group).

Proposed Course:(Cont'd)

2. The medical, nursing and pharmacy staffs will all be instructed in the use of the touch sensitive input-output terminal. Data entries from locations other than the Tumor Service on 4 South, laboratory results, radiographic reports, etc., will be entered by members of the staff.

3. Observations will be made concerning the adaptability of the PROMIS system to various clinical situations encountered in chemotherapy and an attempt will be made to determine its convenience for and acceptance to members of the medical, nursing and pharmacy staff.

4. If feasible, the BCRC staff, with the aid of the PROMIS programmers, will attempt to define research retrieval tools and clinical research patient records with respect to long term accessibility, format, content, syntex, stability, file structure, et. If feasible, an attempt will be made to develop programs which automatically retrieve and analyze data from a number of individual patient records, and compile statistics relating to the results of treatment.

Honors and Awards: None

Publications: None

Lectures: Robert J. Esterhay, Jr., M.D.

1. Good Samaritan Hospital, September, 1971 " The POMP in Rehabilitation Medicine"
2. St. Agnes Hospital, October, 1971 " Problem Oriented Chart".
3. National Institutes of Health, CC/DCRT Coordination Meeting, November, 1971 "Computer Application of the Problem Oriented Medical Record in Medical Oncology"
4. Sinai Hospital, February, 1972 " The POMR: paper/manual (M-POMR) form and computer (C-POMR) form".

Serial No. NCI-6260
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of the Efficacy and Toxicity of Daunomycin in a 180 mg/M^2 Single IV Injection in the Treatment of Acute Nonlymphocytic Leukemia

Previous Serial Number: Same

Principal Investigator: William H. Greene, M.D.

Other Investigators: David Huffman, M.D., Nicholas Bachur, Ph.D., M.D., Stephen Schimpff, M.D., Jerome Block, M.D., and Peter H. Wiernik, M.D.

Man Years

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives:

Daunomycin has been proven to be a potent antileukemic agent in patients with acute nonlymphocytic leukemia utilizing a dosage of 60 mg/M^2 for three successive days. Pharmacokinetic considerations, i.e., the prolonged serum half-life of daunomycin in a single intravenous injection as determined in patients with solid tumors, led to the institution of the regimen under study. The purpose of this study was to determine the toxicity and efficacy of the single dose in patients with acute nonlymphocytic leukemia, both untreated and previously treated. In addition, correlation was made of *in vitro* determinations of myeloblast daunorubicin reductase (which converts daunorubicin to daunorubicinol) with clinical response.

Major Findings:

1. Results - Twenty-three patients received at least one dose of daunomycin at 180 mg/M^2.

Of the 23 patients, 16 were previously untreated and 7 were previously treated with other agents. Among the 16 patients not previously treated, 4 achieved complete remission, 2 achieved a complete bone marrow and hematologic remission, 7 died during induction therapy and 4 had no response. Among

Major Findings (cont'd):

the 7 patients previously treated with other agents, 2 achieved complete remission, one partial remission, one had no response, and one died in induction therapy, and one failed to receive adequate course before being changed to other agents.

2. Toxicity - The major toxicity was marked bone marrow depression, requiring in excess of 10-14 days for recovery. Deaths during therapy were primarily infectious. One patient died with clinical evidence of congestive heart failure and shock, not conclusively linked to a known etiology. Toxicity was greatest in patients $\geq$60 years of age.

3. Metabolic Determinations - Determinations of pre-treatment myeloblast daunorubicin reductase activity were performed in 11 patients and expressed as the ratio of myeloblast to erythrocyte daunorubicin reductase. When these ratios were compared to clinical course, it was found that ratios of <30 were uniformly associated with death during induction therapy (4 patients) or a failure to respond to therapy (3 patients), whereas all those showing bone marrow response (4 patients) had ratios >40. It may be that in vitro determinations of circulating myeloblast daunorubicin reductase activity performed prior to therapy may select out those patients least likely to respond to the drug as administered in this regimen.

Significance to Cancer Research:

Daunorubicin is an effective but toxic agent in acute nonlymphocytic leukemia. The regimen studied here may be useful particularly among patients <60 years of age and in those in whom precautions (LAF, bowel sterilization) are taken to prevent infection. More importantly, the correlation of an in vitro enzymatic determination with clinical outcome may make more efficacious and less toxic the use of this and perhaps other regimens utilizing daunorubicin as the sole antileukemic agent.

Proposed Course: Study completed.

Honors and Awards: None

Publications:

Greene, W., Huffman, D., Schimpff, S., Bachur, N.: Single high dose daunorubicin therapy for acute nonlymphocytic leukemia (ANLL). Proc. Amer. Soc. Clin. Oncol., May, 1972. (Abstract)

Greene, W., Huffman, D., Schimpff, S., Bachur, N.: Single high dose daunorubicin therapy for acute nonlymphocytic leukemia (ANLL). Cancer, 1972 (in press).

Presentations:

I Dr. Greene

1. Single high dose daunorubicin therapy for acute nonlymphocytic leukemia patients. American Society for Clinical Oncology, Boston, Massachusetts, May, 1972.

Serial No. NCI-6261
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Hyperalimentation in Patients with Malignant Disease

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, M.S., R.Ph., William Greene, M.D.

Other Investigators: Robert S. Benjamin, M.D., James C. Cradock, M.S., Lois Jepsen, R.N., B.S.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives:

To assess the usefulness of parenteral hyperalimentation as an adjunct to chemotherapy in the treatment of neoplastic disease.

Methods Employed:

Patients are given intravenous infusions of hypertonic glucose, protein hydrolysate, vitamins, and minerals providing up to 5500 calories daily. The solutions are administered through indwelling catheters placed in the subclavian vein using strict aseptic techniques. Metabolic balance is studied by chemical analysis of collected blood and urine specimens, and body weight is followed as well.

Major Findings:

Hyperalimentation appears to prevent weight loss in patients made anorexic by chemotherapy. Fluid imbalance and infectious complications from the intravenous catheter have occurred in about half the patients, but the incidence has decreased as the staff has gained experience with the technique. Long-term weight gain has been produced in several patients. The administration of high-calorie, high-nitrogen infusions has not resulted in demonstrable acceleration of tumor growth. However, no definite long-term contribution to the well being and survival of any patient has been documented.

Major Findings (cont'd):

Additionally, parenteral hyperalimentation, despite aseptic procedures, has in our hands and those of others been associated with a high incidence of fungemia and disseminated fungal disease.

Significance to Cancer Research:

Despite the theoretical-possibilities that maintaining adequate nutrition in patients with cancer will improve tolerance to chemotherapy, radiation and major surgery, few baseline studies document this idea in the past, using forced feeding regimens. Additionally, there is little recent data reviewing carefully the extent of protein-calorie-vitamin malnutrition and malabsorption in cancer patients. Hence, both the extent of the problem and the value of this regimen are uncertain.

Proposed Course: Discontinue present study in favor of formation of data base as to extent of problem.

Honors and Awards: None

Publications:

Fortner, C.L., Foley, T., and Cradock, J.C.: Reduction in serum CO_2 content during hyperalimentation therapy. Amer. J. Hosp. Pharm. 28:121-122, 1971.

Serial No.: NCI-6263
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

Previous Serial Number: Same

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert S. Benjamin, M.D.

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

1. To improve staging procedures
2. To assess value of "prophylactic" splenectomy
3. To determine if localized disease is curable
4. To evaluate various therapeutic methods for generalized disease

Methods Employed:

Staging to include laparotomy and splenectomy in patients not definitely staged as IV by less invasive procedures. Randomization of patients with stage IV disease to prophylactic splenectomy or not. Randomization of stage I and II patients to radiation with or without subsequent chemotherapy. Randomization of stage III and IV patients to MOPP (HN_2, 6 mg./M^2 IV day 1 & 8, VCR 2 mg. IV day 1 & 8, pred. 40 mg./M^2 qd & 14, and procarbazine 100 mgs. p.o. qd X 14) or COP (CTX, 1500 mg./M^2 day 1, VCR 2 mg. day 1 & 8, pred. 40 mg./M^2 qd X 14). These schedules have been changed somewhat from the originals to make them more comparable with each other and with standard VCR, pred. therapy.

Major Findings:

Laparotomy has been of value in increasing diagnostic accuracy but is not

Major Findings: (Cont'd)

100% accurate. Both drug regimens are effective with complete and partial remission rates of 86% for COP and 87% for MOPP in all patients.

Significance to Cancer Research:

To enhance our knowledge of the nature and extent of untreated lymphomas; to define the therapeutic role of prophylactic splenectomy and to further evaluate 2 combinations of chemotherapeutic agents, one of which has not been thoroughly investigated in this dosage schedule.

Proposed Course: Continue the study.

Honors and Awards: None.

Publications: None.

Serial No. NCI 6264
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Clinical Observations of Infectious Diseases in Cancer Patients

Previous Serial Number: 6264

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: William H. Greene, M.D., Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, Viola M. Young, Ph.D.

Man Years

Total:	5/16
Professional:	1/4
Other:	1/16

Project Description:

Objectives:

Infection is the most common major complication of cancer drug therapy and is a frequent complication in the untreated cancer patient. This investigation is aimed at 1) enumerating the incidence of the various types of infection in cancer patients, 2) describing and analyzing the clinical manifestations of infection, 3) assessing the value of various diagnostic maneuvers, and 4) correlating the site of infection, occurrence of bacteremia, etiologic organism, and clinical presentation and course with the patient's tumor type and defense mechanisms (granulocyte count, corticosteroid therapy, obstruction by tumor, etc.).

Methods:

Each patient with a known or suspected infection is seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records of history, physical findings, nursing notes, laboratory data, and microbiologic isolations are kept. Monthly summaries are prepared and distributed. A detailed summarization of the first full year's observations has been prepared.

Major Findings:

A) Overall Infections - During a 12 month period, July 1, 1970 to June 30, 1971, there were 255 infections in the 305 patients seen at the BCRC. Patients with acute nonlymphocytic leukemia tended to have at least three major infections a piece and infection accounted for 13 of the 20 deaths during that year. Patients with other types of tumor had considerably fewer infections per patient and a smaller percentage of patients died of infection. However, overall, 54 of the 105 deaths during that year were due to infection; of these, 11 were due to Pseudomonas aeruginosa, three to E. coli, five to Klebsiella, seven to Staphylococcus aureus, and three to Candida albicans. The most common sites of infections were lung - 49, skin - 35, urinary tract - 40, pharynx - 15, anorectum - 10, and intestine - 11. Microbiologic documentation of infection was possible in 202 of the 255 clinical infections. Positive blood cultures were found in 70 of these 202 documented infections. It is clear from this information that 1) infection, especially by gram negative organisms, is common and is more prevalent than generally appreciated. 2) Infection can usually be related to the granulocytopenia of cancer drug therapy, but 3) many patients develop infection regardless of the white blood cell level. These infections are usually due to a) tumor obstruction or b) iatrogenic maneuvers (e.g., urinary or intravenous catheters). 4) All patients with cancer are not equally susceptible to all infections but each has a specific set of predisposing factors which, once known, can be useful in predicting the subsequent types of infection which may occur. 5) Infection occurring in the granulocytopenic patient, even when caused by common pathogen, will not necessarily present in the classical manner (e.g., rectal abscesses are watery, pneumonias are less well defined on x-ray, etc.).

B) Acquisition of Potential Pathogens - All patients with acute nonlymphocytic leukemia had cultures of five or more sites taken on admission and then twice weekly thereafter during hospitalization. Using these cultures, we have been able to determine which organisms are part of the patient's normal flora upon admission and which potential pathogens are acquired following admission to the BCRC. Over a two and a half year period of time, 111 clinical infections were recognized, of which 84 had microbiologic documentation. It was found that 47% of these documented infections were due to organisms which had been acquired subsequent to admission to this hospital. Of particular importance was Pseudomonas aeruginosa; nine patients were colonized on admission and three developed bacteremia. Twenty-two patients were colonized following admission and of these, 13 patients developed 16 bacteremias. These hospital-acquired Pseudomonas aeruginosa bacteremias accounted for more than half of the hospital-acquired bacteremias during this time period in acute leukemia patients; it, therefore, follows that methods which will reduce or prevent acquisition of Pseudomonas should reduce the incidence of hospital-acquired bacteremia.

Major Findings (cont'd):

C) Herpes Zoster - A careful investigation has been made of varicella-zoster infections at the BCRC from July 1, 1969 to the present. During the first two years of that study, 39 patients developed 43 episodes of varicella-zoster infection. The investigation showed that varicella-zoster takes four forms of which three represent a secondary form of infection. The infections among the patients at the BCRC occurred primarily in those patients with Hodgkin's disease or other forms of lymphoma and only rarely in patients with leukemia, brain tumor, or metastatic solid tumor. Among the lymphoma patients, it was those patients with advanced disease, cutaneous anergy and recent radiation therapy which had the highest incidence (69%) of developing herpes zoster infection. Epidemiologic evidence was collected to show that herpes zoster, at least in this patient population, is spread by patient-to-patient contact and is not necessarily always a latent infection. This finding implies that patients with advanced Hodgkin's disease who are anergic and who are receiving radiation therapy should have special efforts taken to insure that they will not come in contact with other patients with zoster or with children with varicella.

D) Rectal Abscesses - All episodes of anorectal infection have been carefully documented over the past three years; during this time, 19 episodes of anorectal abscesses have occurred with 16 of these occurring in patients with acute nonlymphocytic leukemia. These infections tend to occur in patients with a prior history of hemorrhoids, who are now granulocytopenic and have been so for some period of time, and who have some recent change in bowel function, be it either diarrhea or constipation. The incidence of rectal abscesses appears to be highest in that group of acute leukemic patients who have monocytic or myelomonocytic leukemia. These infections tend to be difficult to diagnose because of the atypical character of the abscess due to the granulocytopenia. These abscesses accounted for approximately 15% of the microbiologically documented infections in patients with acute nonlymphocytic leukemia during this period of time.

E) BCG Infection - Among the variety of patients treated with BCG as a nonspecific immunostimulant, one patient with acute lymphocytic leukemia while in relapse developed clinical evidence of activation of BCG. This infection was controlled by the use of INH and ethambutal. The observation is instructive because it is a reminder that the patient who has been treated in the past with BCG may have activation of infection, and this must be carefully looked for in such patients who present with fever and adenopathy.

F) Hyperalimentation - During the previous year, 27 patients were treated with hyperalimentation for varying periods of time. Eighteen bacteremias occurred in association with hyperalimentation in these 27 patients. These bacteremias included Candida albicans - 4, Klebsiella sp. - 4, group D streptococcus - 4, and Pseudomonas aeruginosa - 3. Despite the fact that the infection occurred during hyperalimentation, it must also be pointed

Major Findings (cont'd):

out that all of these patients had significant and advanced underlying malignant disease, 16 were receiving cytotoxic chemotherapy, five were on corticosteroids, ten were granulocytopenic with a total count of less than $100/mm^3$ and 15 had had other recent infections treated with systemic antibiotics. Although hyperalimentation per se cannot be implicated in these 18 infections, it is pertinent to note that infections occur frequently in this group of patients and any signs of infection should suggest a bacteremia with removal of the hyperalimentation catheter.

Proposed Course: Continue investigation.

Significance to Cancer Research:

A more complete understanding of the infectious problem in cancer patients is necessarily the first step in adequate prevention and therapy of infection and therefore, in adequate therapy of cancer. Most patients with hematologic malignancies die of infectious complications today.

Honors and Awards: None.

Presentations:

I Dr. Schimpff

1. Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy. October, 1970. Chicago, Illinois.

2. Empiric use of carbenicillin and gentamicin for presumed infection in granulocytopenic cancer patients. Eastern Section of the American Federation for Clinical Research. December, 1970. New York, New York.

3. Epidemic varicella-zoster infection in cancer patients. 52nd Annual Meeting of the American College of Physicians. March, 1971. Denver, Colorado.

4. Pseudomonas infections in cancer patients. Annual Meeting of the American Society of Contemporary Medicine and Surgery. March, 1972. Hollywood, Florida.

5. Infection in patients with cancer. Grand Rounds, Fitzpatrick Mercy Hospital, March 9, 1972, Chester, Pennsylvania.

6. Varicella-zoster infections. Infectious Disease Seminar, University of Maryland, October 10, 1971, Baltimore, Maryland.

Presentations (cont'd):

II Dr. Greene

1. Non-Bacterial infections at autopsy in patients with cancer. American Federation for Clinical Research. April, 1972. Atlantic City, New Jersey.

Publications:

Schimpff, S., Moody, M., and Young, V.M.: Relationship of colonization with Pseudomonas aeruginosa to development of Pseudomonas bacteremia in cancer patients. Antimicrobial Agents and Chemotherapy 10:240-244, 1971.

Schimpff, S., Serpick, A., Stoler, B., Rumack, B., Mellin, H., Joseph, J., Block, J.: Varicella-zoster infection in patients with cancer. Ann Int Med 76:241-254, 1972.

Serial No. NCI-6266
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Therapy of Infection in Patients with Cancer

Previous Serial Number: 6266, 6267

Principal Investigator: Stephen C. Schimpff, M.D.

Other Investigators: William H. Greene, M.D., Peter H. Wiernik, M.D.

Cooperating Units: Research Microbiology, Viola M. Young, Ph.D.

Man Years

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

A) Empiric Antibiotic Therapy for Granulocytopenic, Febrile, Cancer Patients- Infection is a frequent and serious complication in the granulocytopenic patient with cancer. Bacteremia commonly occurs and usually leads to rapid demise. This investigation was designed to assess the efficacy of systemic antibiotics, given as initial empiric therapy, for the therapy of presumed sepsis in granulocytopenic, cancer patients.

B) Use of Cytosine Arabinoside in the Therapy of Varicella-Zoster Infections- Because zoster infections at this cancer center are common and because they have a significant degree of morbidity, and because there is preliminary evidence to suggest that cytosine arabinoside will halt the multiplication of varicella-zoster virus, it was decided to use cytosine arabinoside as systemic therapy of patients with early zoster in a controlled, randomized trial to determine whether this therapy would be better than placebo in halting the progression of infection.

C) Studies with 5-Fluorocytosine for Fungal Infections in Patients with Cancer- Fungal infections are frequent in patients at this cancer center. They are frequently not diagnosed during life but a high number of infections has been detected at autopsy. The only other available agent for the treatment of systemic fungal infection is Amphotericin B which has a high degree of toxi-

Objectives (cont'd):

city. In vitro studies show that 5-fluorocytosine is active against Cryptococcus and Candida albicans in most cases. Five-fluorocytosine has, therefore, been used in selected patients with proven or strongly suspected clinical evidence of fungal infection.

D) Initial Studies of a New Antibiotic Tobramycin - Tobramycin, an aminoglycoside similar to gentamicin, has a spectrum of activity similar to gentamicin. However, in vitro studies suggest that it is two to four times more active than gentamicin against Pseudomonas aeruginosa. Pseudomonas aeruginosa is the most common cause of significant and serious infection at the BCRC. It was, therefore, decided that Tobramycin should be intensively studied at this Center.

Major Findings:

A) Empiric Antibiotic Therapy - The initial eighteen month study with carbenicillin and gentamicin used to treat 75 patients showed that 48 patients had subsequent proof of infection. Of these 48 patients, 28 improved, 15 did not, and five could not be evaluated. Twenty-one patients had Pseudomonas aeruginosa infection; 80% improved. Results against infections due to other gram negative organisms were not as good. For this reason that study was terminated and a new study using carbenicillin, gentamicin plus cephalothin was begun. That study has now been in progress for approximately 12 months, and it should be possible shortly to determine whether the addition of cephalothin has increased the efficacy of this combination against the common causes of gram negative rod infection in this patient population.

B) Cytosine Arabinoside for Herpes Zoster - In a pilot study of four patients with varicella-zoster infection, it was believed that three patients showed no further progression of infection. The controlled trial is now in progress, but it is too early for evaluation.

C) 5-Fluorocytosine - Although 5-fluorocytosine can be taken orally and no major toxicities have been encountered, in the few patients in whom it had been used, there have been few marked responses to this form of therapy for systemic fungal infections.

D) Tobramycin - Studies of Tobramycin have only just begun; however, a carbenicillin-gentamicin resistant Pseudomonas aeruginosa which was infecting a patient was found to be susceptible to Tobramycin.

Proposed Course: Continue all studies.

Significance to Cancer Research:

The empiric use of systemic antibiotics for presumed bacterial sepsis has significantly reduced the morbidity and mortality of Pseudomonas aeruginosa sepsis and as such, has permitted prolongation of life so that more adequate cancer chemotherapy could be used.

Honors and Awards: None

Presentations:

I Dr. Schimpff

1. Empiric therapy with carbenicillin and gentamicin for febrile, granucytopenic cancer patients. Eastern Section of the American Federation for Clinical Research, New York, New York. December, 1970.

2. Use of carbenicillin in cancer patients. Film presentation made to representatives of Roerig Division of Pfizer Corporation, December 18, 1971.

3. Carbenicillin-gentamicin therapy of cancer patients. Grand Rounds, Veterans Administration Hospital, Baltimore, Maryland, March, 1971.

Publications:

Schimpff, S., Satterlee, W., Young, V.M., and Serpick, A.: Empiric therapy with carbenicillin and gentamicin for febrile, granulocytopenic cancer patients. New Eng J Med 284:1061-1065, 1971.

Serial No.: NCI-6276
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Chemotherapy of Metastatic Testicular Carcinoma

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert Esterhay, Jr., M.D.
Michael J. O'Connell, M.D.
David Cook, M.D.
Edward Sickles, M.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives:

To access the efficacy of various anti-tumor chemotherapeutic agents in metastatic testicular carcinoma.

Methods Employed:

Patients are currently randomized between a combination of drugs consisting of fluorouracil, cyclophosphamide, methotrexate, and vincristine and a combination including the same drugs plus bleomycin given 12 hours after the dose of vincristine.

Major Findings:

Of 10 patients entered into this study, 5 on each arm, 9 patients have shown substantial, clinically useful response. The numbers are still too small to determine whether or not one arm of this treatment protocol is superior to the other. Toxicity has been remarkably minimal.

Significance to Cancer Research:

Testicular carcinomas account for a significant proportion of tumors in young male adults. Metastatic testicular carcinoma has been shown to be responsive to several different classes of anti-tumor agents by this group and others. The present study shows promise of achieving results superior to those previously reported.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications: None

Serial No.:NCI-6277
1. BCRC
2. Medicine Section
3. Baltimore,Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Chemotherapy of Adult Acute Leukemia

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: William H. Greene, M.D.
Stephen C. Schimpff, M.D.
Robert S. Benjamin, M.D.
Nicholas R. Bachur, M.D.

Cooperating Units: Acute Leukemia Cooperative Group B
Research Microbiology, BCRC
Patient Care Pharmacy, BCRC
Section of Biochemistry, Laboratory of Pharmacology,BCRC
Section of Neurological Surgery, BCRC

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description:

Objectives:

1) To evaluate the effectiveness of newer chemotherapeutic agents alone and in combination against adult acute leukemia.
2) To improve the efficacy of anti-leukemic agents of proven activity by manipulating the schedule of administration.
3) To correlate clinical results with clinical pharmacokinetic data.
4) To identify factors of prognostic import in adult acute non-lymphocytic leukemia.
5) To study the effect of intensive anti-leukemic therapy during complete hematologic remission.
6) To identify biochemical indicators of occult disease activity in adult patients with acute non-lymphocytic leukemia.

Methods Employed:

Protocol studies of various anti-tumor agents alone or in combination have been initiated. In addition muramidase has been measured by a turbidimetric

Methods Employed: (Cont'd)

method, carcinoembryonic antigen by radioimmunoassay, and immunoglobulin by immunoelectrophoresis.

Major Findings:

I. Effect of Single Agents

Daunomycin:

Daunomycin (Daunorubicin) was given to 22 adults with acute non-lymphocytic leukemia in a new dosage and schedule suggested by pharmacokinetic data from the Section of Biochemistry: 60 mgs. per sq. meter of body surface area daily injection for 3 days. A complete remission rate of 50% was obtained with this regimen in patients whose median age was 48 years. The duration of remission in this group of patients was 2.3 months median with a range of 1-11.5 months. The median survival of remitting patients was 12 months with a range of 5-24 months as compared to those who failed to achieve a complete remission in which the median survival was 1 month.

II. Effect of Multiple Drugs in Combination

A) POMP:

21 adults with acute non-lymphocytic leukemia with a median age of 53 years were treated with a combination of prednisone, vincristine, mercaptopurine, and methotrexate in large doses. A complete remission was achieved in 28% of patients and the median age of the responders was 6 months with a range of 1-12 months. The median survival of remitting patients was 11 months with a range of 5-19 months. This compares favorably with the median survival of non-responders which was less than 1 month.

B) Daunomycin, Cytosine Arabinoside, Thioguanine and Pyrimethamine:

This program was undertaken to investigate whether or not a combination of proven anti-leukemic agents might induce a complete remission in a higher percentage of adult patients with acute non-lymphocytic leukemia than is presently achieved with single agents or other combinations. In addition pyrimethamine is included in this study in an attempt to treat occult central nervous system leukemia at the time of diagnosis with an agent that can be given orally. As a control for this study our best single agent in our best schedule, that is, daunomycin 60 mgs. per sq. meter daily for 3 days, was used. Patients are randomized between the two arms at the time of admission. To date the remission rate for patients receiving the combination is 70% and the rate for those receiving daunomycin alone is 50%. Thus far, the incidence of meningeal leukemia in the combination group is distinctly less than that of the daunomycin alone group. However numbers are too small for statistical evaluation at this point.

III. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia

A) Cytoxan and Guanozole:
All patients achieving complete remission on the daunomycin alone arm or the daunomycin, cytosine arabinoside, thioguanine and pyrimethamine arm of our protocol 7110 are maintained with monthly injections of cytoxan 1500 mgs. per sq. meter of body surface area followed in 18 hours by guanozole 20 grams per sq. meter in a 24 hour I.V. infusion. It is hoped that by possibly cycling leukemia cells with cytoxan and then attacking them with the cell cycle specific agent, guanozole, a significant cell kill might be achieved during a period of hematologic and clinical remission, thereby prolonging the duration of remission over that which is customarily achieved. It is too early to evaluate the results of this study.

IV. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia.

A) Serum and Urine Muramidase Studies:
Our studies in serum and urine muramidase activity in adults with acute non-lymphocytic leukemia continue. We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B) Carcinoembryonic Antigen:
We have observed that approximately 20% of adults with acute non-lymphocytic leukemia have abnormal levels of carcinoembryonic antigen in their serum. It is not known at this time whether or not fluctuations in carcinoembryonic antigen serum concentration reflect disease activity in adult acute non-lymphocytic leukemia.

C) Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia:
We have measured quanitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved these levels become normal. Investigation continues into the question whether or

C) Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia: (Cont'd)

not serial immunoglobulin levels might be useful in determining the disease status in a patient with acute non-lymphocytic leukemia.

Summary of Major Findings:

1. Combinations of effective anti-tumor agents may induce remission in a higher percentage of patients with adult acute non-lymphocytic leukemia than single agents do.
2. Serum muramidase levels are useful in following disease activity in adult acute non-lymphocytic leukemia.
3. Carcinoembryonic antigen is elevated in a small percentage of adults with acute non-lymphocytic leukemia and serial determinations may be useful in accessing disease activity.

Significance to Cancer Research:

These studies indicate continued improvement in our ability to arrest disease activity in adults with acute non-lymphocytic leukemia in an ever increasing number of patients. In addition biochemical parameters are being identified that reflect accurately disease activity in many patients with acute non-lymphocytic leukemia. The identification of such factors may lead to more rational therapy in the future.

Proposed Course of Project:

These investigations will be continued through the fiscal year.

Honors and Awards: None.

Invited Lectures: Peter H. Wiernik, M.D.

1) Johns Hopkins University School of Medicine -	July, 1971 January, 1972
2) University of Maryland School of Medicine -	July, 1971
3) University of Virginia School of Medicine -	July, 1971
4) Harbor General Hospital, Los Angeles -	September, 1971
5) Bowman Gray School of Medicine -	October, 1971
6) Western Reserve University School of Medicine -	November, 1971
7) Emory University School of Medicine -	December, 1971
8) University of Miami, School of Medicine -	January, 1972
9) Bon Secours Hospital, Baltimore, Maryland -	April, 1972
10) Greater Baltimore Medical Center -	April, 1972
11) VA Hospital, Washington, D.C.	January, 1972
12) Visiting Professor; University of Minnesota, School of Medicine, Minneapolis, Minnesota -	August, 1971
13) Invited speaker,"Topics in Clinical Medicine", Johns Hopkins Hospital-	May, 1972

Invited Lectures: Peter H. Wiernik, M.D. (Cont'd)

14) Invited Speaker, "Advances in Cancer Therapy", Joint Meeting Clin. Soc and Officer's Assoc., U.S.Public Health Service Hospital - May, 1972

Publications:

1. Wiernik, P.H. and Duncan, J.H.: Cyclophosphamide in Human Milk. Lancet, 1:912, 1971.
2. Smith, K.A., Wiernik, P.H., and Walker, M.D.: The Cytocentrifuge in the Diagnosis of CNS Leukemia. Proc. Amer. Soc. Hematology, 1971.
3. Wiernik, P.H. and Serpick, A.A.: Single Agent or Combination Therapy for Remission Induction in Adult Acute Non-Lymphocytic Leukemia ? A Randomized Clinical Trial of Daunorubicin and "POMP". Cancer Research, In Press.
4. Wiernik, P.H.: Carcinoembryonic Antigen (CEA) in Hematologic Malignancies. Proc. XIV Int. Cong. Hematology, 1972.

Serial No.: NCI-6278
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Determination of Serum Alpha Fetoprotein and Plasma Carcinoembryonic Antigen in Patients with Malignant Disease.

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Robert Esterhay, Jr., M.D.

Cooperating Units: Laboratory of Cell Biology, NCI
Hoffmann-La Roche, Medical Research Department, Nutley, New Jersey
Section of Neurological Surgery, BCRC

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To determine whether or not carcinoembryonic antigen and alpha fetoprotein are present in the serum or plasma of patients with a variety of malignant diseases. In addition to determine whether or not serial determinations of alpha fetoprotein and carcinoembryonic antigen in patients with initially elevated levels are useful in following disease activity.

Methods Employed:

Carcinoembryonic antigen and alpha fetoprotein are determined by radioimmunoassay. Patients are investigated prior to any therapy and those with positive results are followed serially throughout their course.

Major Findings:

1. Carcinoembryonic antigen is present in the plasma of patients with a variety of neoplasms including hematologic malignancies, brain tumors, neuroblastoma, gastrointestinal tumors and others.
2. In some patients plasma carcinoembryonic antigen levels accurately reflect disease activity.

Major Findings:(Cont'd)

3. Alpha fetoprotein elevations in sera are not specific for patients with hepatocellular carcinoma. Elevated alpha fetoprotein is found in about 25% of patients with embryonal cell carcinoma of the testes and teratocarcinoma of the ovary. In addition a rare patient with gastric carcinoma with elevated serum alpha fetoprotein can be identified.

Significance to Cancer Research:

The identification of biochemical parameters that may signal the presence of occult tumors is of prime importance to optimal therapy of cancer.

Proposed Course:

Continue present studies.

Honors and Awards: None.

Publications:

Mehlman, D.J., Bulkley, B.H., and Wiernik, P.H.: Serum Alpha Fetoprotein with Gastric and Prostatic Carcinomas. New Eng. J. Med., 285:1060, 1971.

Serial No.: NCI-6279
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Initial Clinical and Pharmacological Evaluation of Adriamycin

Previous Serial Number: None

Principal Investigator: Robert Benjamin, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Nicholas Bachur, M.D.

Cooperating Units: Biochemistry Section, Laboratory of Pharmacology, BCRC

Man Years:

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives:

1. To determine the effectiveness and toxicity of Adriamycin given by a single intermittent high-dose schedule in a variety of malignancies.
2. To study the pharmacokinetics of Adriamycin in these patients.
3. To make clinical and pharmacologic correlations.

Methods Employed:

Any patient with metastatic cancer for which no better therapy is available is elligible for the study. Adriamycin is given in a dosage of 60mg/M^2 and repeated in 3-4 weeks after toxicity from the previous dose has resolved. Serial plasma and urinary samples are to be collected, extracted with acid alcohol, and analyzed for drug and metabolite.

Major Findings:

I. Clinical

Adriamycin is an effective drug against a wide variety of solid tumors and acute lymphocytic leukemia in this dosage regimen. In the presently evaluable patients a complete remission was obtained in 1/2 of patients

Major Findings: (Cont'd)

I. Clinical (Cont'd)

with acute lymphocytic leukemia. Partial responses have been obtained in osteogenic sarcoma, rhabdomyosarcoma, leiomyosarcoma, reticulum cell sarcoma, lung carcinoma, and neuroblastoma. The incidence of mucositis has been substantially lower than in previously reported series, presumably related to the dosage schedule. Severe hematologic toxicity correlated with the presence of liver disease or bone marrow infiltration with tumor.

II. Pharmacokinetics

A mean plasma half life of 27 hours and relative volume of distribution of 246 l/M^2 has been determined in 14 patients without hepatic insufficiency. Total 5 day urinary excretion is only 5.5% of the administered dose.

III. Clinical Pharmacologic Correlations

A patient with renal insufficiency had normal Adriamycin plasma half-life and reasonable toxicity. Patients with hepatic insufficiency have prolonged Adriamycin plasma half-lives due to the delayed appearance of fluorescant metabolites. These patients had severe toxicity.

Significance to Cancer Research:

This is a new anti-tumor agent that we have shown to be effective in a variety of human neoplasms. Studies of the distribution of the drug within the host, and of the mechanics by which the drug is excreted will hopefully lead to more effective schedules and doses of this agent.

Proposed Course:

Continue the study.

Honors and Awards: None

Publications:

Robert S. Benjamin, David H. Huffman, Peter H. Wiernik, and Nicholas R. Bachur: Pharmacokinetics of Adriamycin (A) in Man, Proc. Amer. Assoc. Cancer Res. 13:115, 1972.(Abstract)

R.S. Benjamin, P.H. Wiernik, D.H. Huffman and N.R. Bachur: Clinical and Biochemical Studies of Intermittent Adriamycin (A) and Daunorubicin (D) Dosage Schedules in Solid Tumor Patients, Clin. Res.,20:563, 1972.(Abstract)

Publications: (Cont'd)

Robert S. Benjamin, David H. Huffman, Peter H. Wiernik and Nicholas R. Bachur: Pharmacokinetics and Metabolism of Adriamycin (A) in Patients with Hematologic Neoplasma and Other Malignancies, XIV Int.Cong.Hematology (For presentation) In Press.

Serial No.: NCI-6280

1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Combination Chemotherapy for Stage III-B and IV Hodgkin's Disease

Previous Serial Number: None

Principal Investigators: Peter H. Wiernik, M.D.

Other Investigators: Robert S. Benjamin, M.D.
Michael J. O'Connell, M.D.
Edward A. Sickles, M.D.
David Cook, M.D.
Donald Sklansky, M.D.
Richard S. Kaplan, M.D.

Man Years:

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine whether a combination of cyclophosphamide, vinblastine, procarbazine, and prednisone might prove efficacious in the therapy of advanced Hodgkin's Disease.

Major Findings:

1. Thus far 17 patients with advanced Hodgkin's Disease have been placed on this study. All have shown significant response to the drug regimen.
2. Toxicity has been severe in about 1/3 of patients making necessary dosage and scheduling alterations.

Significance to Cancer Research:

Although many combinations of effective agents have been identified for the treatment of advanced Hodgkin's Disease, in the best hands 20% of patients fail to respond significantly. If this study will result in a regimen that produces complete response in a greater number of patients and a longer

Significance to Cancer Research: (Cont'd)

duration of response than obtained with other regimens, the therapy of disseminated Hodgkin's Disease will have been improved.

Proposed Course:

Continue present study.

Honors and Awards: None.

Publications: None.

Presentations: Peter H. Wiernik, M.D.
Hematology Division Seminar
Department of Medicine
Johns Hopkins University School of Medicine
December, 1971

Serial No.: NCI-6281
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Initial Clinical Trials of Streptozotocin in the Treatment of Metastatic Cancer

Previous Serial Number: None

Principal Investigators: Peter H. Wiernik, M.D.
Michael J. O'Connell, M.D.

Other Investigators: Edward Sickles, M.D.
David Cook, M.D.

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives:

To determine whether or not Streptozotocin is a useful therapeutic agent in human neoplastic diseases.

Methods Employed:

Patients are treated with intravenous Streptozotocin on a weekly basis with doses that have been determined to be associated with clinically acceptable toxicity from earlier Phase I studies.

Major Findings:

To date 11 patients with a variety of tumors have been treated with Streptozotocin. Biochemical parameters have improved in one patient with malignant carcinoid and in one patient with neuroblastoma. Only one patient showed evidence of clinically useful response, however.

Significance to Cancer Research:

Streptozotocin is an agent of potential therapeutic value that is not significantly myelosuppressive. Therefore it would be an important adjuvant to our chemotherapeutic armamentarium if significant anti-disease activity could be identified.

Proposed Course:

Continue present studies.

Honors and Awards: None

Publications: None

Serial No.: NCI-6293
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS - NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Teaching And Consultation Service in Medical Oncology

Previous Serial Number: None

Principal Investigator: Peter H. Wiernik, M.D.

Other Investigators: Stephen C. Schimpff, M.D.
William Greene, M.D.
Robert Esterhay, Jr., M.D.

Cooperating Units: University of Maryland School of Medicine
Johns Hopkins University School of Medicine

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To teach the theory and practice of medical oncology to medical students and house officers. To acquaint such individuals with newer aspects of chemotherapy, radiotherapy and surgery as they apply to neoplastic diseases. To provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area free of charge.

Methods Employed:

Second year medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition house officers from some private hospitals have been assigned here for elective periods of their house officer year.

Physicians in the community refer patients here for an out-patient consultation. If the patient meets the qualifications for one of our protocol studies he is accepted in transfer and admitted to the BCRC. If not the patient is completely evaluated as an out-patient and returned to his physician

Methods Employed: (Cont'd)

with specific recommendations for therapy.

Major Findings:

1. Students and houseofficers who have rotated through the BCRC are recognizing that medical oncology is a bonafide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care in order to achieve good results.
2. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

Significance to Cancer Research:

The dissemination of information concerning the latest techniques in diagnosis of therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at early time, such as during their student years and house officer years such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions which we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

Proposed Course:

Continue project.

Honors and Awards: None

Publications: None

Lectures: None

Serial No.: NCI-6021
1. Baltimore Cancer Research Center
2. Neurosurgical Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Mechanism of Hydroxylation of Foreign Compounds.

Prevous Serial Number: SAME

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: Barry H. Rumack, M.D.

Project Description:

This project was completed 6/30/71.

Publications:

J. L. Holtzman and B. H. Rumack: The Kinetics of Ethylmorphine Activation of the NADPH Cytochrome P-450 Reductase Activity of Hepatic Microsomes from Male and Female Rats. Chemic-Biol. Interactions, 3: 279-281, 1971.

J. L. Holtzman and D. S. Alberts: The Estimation of Pentobarbitol Hydroxylase Activity by Gas-Liquid Chromatography. Anl. Biochem., 43: 48-56, 1971.

J. L. Holtzman and B. H. Rumack: The Kinetics of Cytochrome P-450 Reductase Stimulation by Ethylmorphine. Life Sciences, 10: 669-677, 1971.

Serial No.: NCI-6022
1. Baltimore Cancer Research Center
2. Neurosurgical Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Effect of Various Factors on the Hepatic Microsomal System and Associated Metabolic Pathways.

Previous Serial Number: SAME

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: Barry H. Rumack, M.D.

Project Description:

This project was completed 6/30/71.

Publications:

J. L. Holtzman, Vicki Rothman and Simeon Margolis: Metabolism of Drugs by Isolated Hepatocytes. Biochem. Pharmacol., 21: 581-584, 1972.

Serial No.: NCI-6024
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of the Neurosurgical Service With the Primary Interest in the Treatment of Brain Tumors at the BCRC, NCI

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Richard Kaplan, M.D.
Donald Sklansky, M.D.
Mark L. Rosenblum, M.D.
Arden F. Reynolds, Jr., M.D. (USPHS)

Cooperating Units: Medical Service, BCRC, NCI
Department of Surgery, USPHS Hospital
Baltimore, Maryland

Man Years:

Total:	1.6
Professional:	1.6
Other:	0.0

Project Description:

Objectives:

This ongoing project is designed to develop an effective research neurosurgical service to evaluate various modalities of treatment of intracranial neoplasm. Pilot studies are performed to evaluate the efficacy of drug in relation to Phase I - II and neuropharmacologic studies. Neurosurgical consultation and expertise are provided the USPHS Hospital, Baltimore, and diagnostic and therapeutic services are provided. Consultation and supervision of NCI projects related to the treatment of malignancy within the intracranial space are made available. Interdisciplinary studies of blood brain barrier, pharmacology and cellular membrane permeabilities are undertaken.

Methods Employed:

Patients with diagnosed intracranial neoplasm are referred to the BCRC, Section of Neurological Surgery, for evaluation and chemotherapy both from within the private community, as well as the PHS system. Primary unoperated upon CNS tumor is preferred, so that pharmacologic evaluation of chemothera-

Methods Employed cont.

peutic agents, intracranial markers, etc., may be carried out as part of the operative procedure. Complete care on an out and inpatient basis, as well as utilization of the intensive care unit and blood component therapy services are available. Due to the potential toxicity of drugs employed, close monitoring of patients is a necessity. Studies utilizing CCNU, Epipodophyllotoxin and Methyl CCNU have been undertaken and the services are participants in the Brain Tumor Study Group.

Frequent consultations and neurologic evaluation of BCRC patients are provided the medical staff to assist in the general medical oncology program. A particular interest in CNS leukemia, as well as metastasis of other tumors, is pursued. CSF clearance of inert substances has been carried out.

In consultation with the Departments of Medicine, Surgery and Neurology of USPHS Hospital, Baltimore, neurosurgical evaluation, neurodiagnostic procedures and surgical intervention for general neurosurgical disease are provided. Consultations on difficult diagnostic problems are undertaken. Thru the Director, surgical and parasurgical communications are maintained. Formal and informal consultations are provided to the greater Baltimore medical community in relation to brain tumor therapy. Informal discussions, telephone consultations and formal lectures are given.

Major Findings:

During the fiscal year there were 148 neurosurgical admissions, 592 outpatients visits were undertaken and 68 consultations were provided. A Phase II study of CCNU has been completed demonstrating approximately 40% complete or partial remission of symptomatology. Epipodophyllotoxin (PTG) has been similarly studied in a Phase II study; this substance appears less promising although some remissions have been noted. Methyl CCNU Phase I and pharmacologic studies have been performed. The maximally tolerated dose has been identified and pharmacologic parameters elucidated (see specific studies). Careful patient monitoring prompts attention to early signs of complications and high quality patient care have been provided to this patient population.

Consultation service within the hospital, the City of Baltimore, nationally and internationally has been provided. Lectures, papers and discussions, have been undertaken within the neuroscience community in order to effectively display the NCI's programs.

CSF space perfusions in monkeys have defined the tolerable doses of 8-Azaguanine at 0.125 and 0.625mg/ml. CSF pharmacologic characteristics have been demonstrated; preliminary studies of Thiotepa have been undertaken. The Rouse sarcoma induced puppy brain tumor has been used to evaluate epipodophyllotoxin, cytoxan and Methyl CCNU at various dose levels and efficacy of the nitrosourea shown.

Significance to Biomedical Research and Program of the Institute:

The incidence of primary central nervous system neoplasm is greater than that of Hodgkins Disease. With the increase in longevity brought about by more successful therapy of cancer elsewhere, cerebral metastasis is becoming more apparent and must be dealt with. The development of a study unit to evaluate the efficacy, toxicity and neuropharmacology of drugs used to treat these disease processes is essential to the conquest of the cancer program.

Proposed Course:

This service will continue to admit, diagnose and treat operatively and chemotherapeutically patients with intracranial malignancy. Phase I studies of new chemotherapeutic agents with appropriate pharmacokinetic characteristics for successful brain tumor chemotherapy will be undertaken. Phase II studies will be undertaken utilizing Methyl CCNU alone and in combination both intravenously and orally. Continued participation in the Brain Tumor Study Group will be undertaken.

Honors and Awards: NONE

Publications:

Walker, M.D. and Rosenblum, M.L.: Treatment of Malignant Brain Tumors with Nitrosoureas. Cancer Chemotherap. Rep. 55 (2): 100, April, 1971.

Henkin, R.I., Fontana, A.J., and Walker, M.D.: On the Mechanism of the Presence and Distribution of Adrenal Corticosteroids in the Central and Peripheral Nervous System. Excerpta Medica International Congress Series No. 219, 1971 on Hormonal Steroids, Proc. of the Third International Congress, Hamburg, September 1970. pp. 792-805

Walker, M.D. and Henkin, R.I.: Blood-Brain Barrier Considerations of Corticosteroids. Psychopharmacology Bull. 8: 5-6, January 1972.

Walker, M.D.: Physiologic and Pharmacologic Aspects of Barbiturates in Elasmobranchs. Comp. Biochem. Physiol. (in press)

Presentations:

American Academy of Neurology, New York. "Malignant Central Nervous System Tumor and Other Neoplasms in a Large Family," April, 1971.

Second Conference on Current Investigations Dealing with Elasmobranch Biology, Bar Harbor, Maine, "Physiologic and Pharmacologic Aspects of Barbiturates in Elasmobranchs," June, 1971.

Research Society of Neurological Surgeons, New York, talk on "Research in Intraarterial Chemotherapy," October, 1971.

Presentations con't

Clinical Congress of The American College of Surgeons, Atlantic City, panelist on the subject of "Diagnosis and Treatment of Pontine Gliomas," October 20, 1971, and Discussant on "An Invetral Assay System for Comparing Isotopic Uptake of Brain Tumor Screening Agents." October 21, 1971.

Nairobi, Kenya: Invited to participate in the Pan African Symposium on Tumours of the Nervous System in the African. Presented talk on "Chemotherapy of Primary Brain Tumors," January 21, 1972.

USPHS Hospital, Baltimore, Medical Record Librarian School. Lecture on "A Survey of Neurosurgical Disease," March 14, 1972.

Johns Hopkins Hospital Neurosurgical Staff. Talk on "Recent Advances in Treatment of Brain Tumors," April 12, 1972.

American Academy of Neurology, St. Louis, Mo. Discussant on paper "Association of Breast Cancer Soft Tissue Sarcoma, Leukemia, and Brain Tumors in Breast Cancer Families," April 28, 1972.

American Association for Cancer Research Meeting, Boston. Talk on "An Evaluation of 1-3-Bis(2-Chloroethyl)-1-Nitrosourea (BCNU) and Irradiation Alone and in Combination for the Treatment of Malignant Glioma," May 5, 1972.

Serial No.: NCI-6202
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Murine Intracerebral Melanoma Study

Previous Serial Number: SAME

Principal Investigator: Donald S. Bowie, B.S.

Other Investigators: Michael D. Walker, M.D.
Roland Williams, M.D. (USPHSH)

Cooperating Units: NONE

Man Years:

Total:	.4
Professional:	.2
Other:	.2

Project Description:

Objectives:

The major objectives of this project are to continue the study of the efficacy of various chemotherapeutic agents against the murine intracerebral melanoma, and also the interaction of other drugs and chemotherapeutic agents against this tumor system.

Methods Employed:

B-16 melanoma of the Jackson Laboratory, Bar Harbor, Maine, has been employed for serial transplant. Varying doses of BCNU, CCNU, and MeCCNU have been used.

Major Findings:

BCNU therapy at 31mg/kg (LD_{10}) increased the life-span of intracranial tumor bearing mice 30% as compared to untreated controls. CCNU in various doses ranging from 33-40mg/kg increased life-span from 21-60%. Early evlauation of MeCCNU in doses ranging from 20-30mg/kg produced increased survival of from 46-72% at the 1.5 LD_{10}.

Proposed Course:

Further studies of this model will be pursued utilizing these drugs to

Proposed Course cont.

investigate two phenomena. Drug-drug interaction with commonly utilized substances will be pursued as well as drug irradiation potentiation in this mouse system. Continued studies of different vehicles for the administration of aqueous insoluble nitrosoureas will be undertaken.

Honors and Awards: NONE

Publications: NONE

Serial No.: NCI-6203
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Drug Distribution in Patients Receiving Therapeutic Doses of CCNU. (Phase II)

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: V.T. Oliverio, Ph.D., V.T. DeVita, M.D.
M.L. Rosenblum, M.D. and A.F. Reynolds, Jr., M.D.
Donald M. Bowie, B.S.

Cooperating Units: Medical Service, NCI, NCI-LCP

Man Years:

Total:	.5
Professional:	.3
Other:	.2

Project Description:

Objectives:

This project is designed to evaluate the pharmacologic absorption, distribution and excretion of antineoplastic agents in patients receiving therapeutic doses of CCNU.

Methods Employed:

Patients receiving CCNU as part of their therapeutic regimen for the treatment of brain tumor are given tracer doses of radio-labeled C^{14} CCNU. Samples of blood, urine, cerebral spinal fluid and tissues are obtained. Patients undergoing surgery for tumor are of particular interest and also receive tracer doses of CCNU in order to analyze the entrance of radio-label and parent compound into tumor, muscle and adjacent brain. Standard radioisotopic measuring techniques are utilized.

Major Findings:

All three labels of CCNU C^{14} (carbonyl, cyclohexyl, and chloroethyl) have been evaluated after administration by mouth. Absorption of the drug is modest and slower than would be expected for a lipid soluble compound. Less than 25% of the drug appearing in plasma is lipid extractable. Significant

Major Findings cont.

amounts of radio-label are found in CSF, brain, muscle and tumor, although the plasma half-life is considerably prolonged. Over 3/4 of the administered dose is accounted for by urinary excretion.

Phase II study of CCNU in the treatemnt of patients with malignant brain tumor has been completed and indicates that 130mg/m^2 of CCNU is well-tolerated. First doses of 150mg/m^2 can also be administered, however, the depth and duration of thrombocytopenia appear to be greater. Therapeutic response was noted in 40% of the patients treated with CCNU, and, therefore, it is considered an active drug in the treatment of malignant brain tumor.

Significance to Biomedical Research and Program of the Institute:

CCNU is the second most carefully studied nitrosourea produced by the NCI programs. Understanding of its pharmacology and entrance into tumor and normal cells will provide information necessary to design better studies and therapeutic regimens.

Proposed Course:

The Phase II study and pharmacologic evaluation of CCNU has been completed. Manuscripts are being prepared for publication.

Honors and Awards: NONE

Publications: NONE

Presentation:

American Association for Cancer Research Meeting, "The Treatment of Brain Tumor with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)."

Serial No.: NCI-6204
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Toxicity of Intracerebral Chemotherapy in Monkeys

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Dal Dalgard, D.V.M.

Cooperating Units: Hazelton Laboratories, Falls Church, Va.

Man Years:

Total:	.5
Professional:	.5
Other:	0.0

Project Description:

Objectives:

With the increase in meningeal leukemia and the interest in neoplasms within the central nervous system, the use of intrathecal chemotherapy is expected to increase. In this ongoing project, a relatively standardized technique for evaluating intrathecal chemotherapy in monkeys is being employed.

Methods Employed:

Intracisternal installation of carefully chosen drugs are first performed to determine acute immediate toxicity. From this information dose levels for chronic studies simulating the dose schedules used in man are developed. Evaluation of activity in indices, hematologic parameters and cerebral spinal fluid are made. Histopathologic review is performed on the upper cervical cord, brain stem and brain.

Major Findings:

Thiotepa has been evaluated at 5 dose levels in a chronic biweekly 8 dose schedule. 3-6mg/m^2 was well-tolerated and only moderate leukopenia without CSF changes was found. Hydroxyurea was evaluated in acute studies and the commercial preparation found to be toxic. A special more nearly neutral formulation was investigated and found to be well-tolerated. Chronic studies at 3 dose levels showed that up to 1200mg/m^2 could be

Major Findings cont.

employed. Procarbazine was evaluated in acute studies and found also to be highly toxic. A special formulation is now being made. Due to concern related to the preservatives Methyl and proparabenes in some drugs, a pilot study using 10 times the amount normally found in preparations given to man was conducted. No untoward effects have been noted, and more detailed study of this will be undertaken.

Significance to Biomedical Research and Program of the Institute:

The use of intrathecal drug in treating human neoplasm is limited to methotrexate and cytosine arabinoside. Developing a body of toxicologic information concerning other drugs placed in the CSF pathways would provide a central core for expanding intrathecal drug usage.

Proposed Course:

Three to four new drugs will be added to this study this year. Special studies related to the preservatives used for intrathecal medication will be continued, and new studies to evaluate physical chemical limits (i.e., osmolality, pH, iron content, etc.) will be undertaken.

Honors and Awards: NONE

Publications: NONE

Serial No.: NCI-6268
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Radio-labeled Distribution of Methyl CCNU in Dogs

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Arden F. Reynolds, Jr., M.D.
Mark L. Rosenblum, M.D.
Donald M. Bowie, B.S.

Cooperating Units:

Man Years:

Total:	.7
Professional:	.4
Other:	.3

Project Description:

Objectives:

This project is designed to determine the pharmacologic characteristics of Methyl CCNU in the dog as preparation for similar determinations in humans. Since Methyl CCNU is aqueous and insoluble, a parental formulation technique is being developed and evaluated in the dog.

Methods Employed:

Following intravenous or intragastric administration of C^{14} MeCCNU labeled in the chloroethyl and methylcyclohexyl moieties, serial blood, CSF, urine, bile, muscle and brain samples have been obtained from 4 minutes up to 24 hours. Direct sample counting, as well as extraction and chromatography, have been carried out. Utilizing different substances pharmacologic acceptability of various vehicles is determined. Fat emulsion appears satisfactory for deliverence of this drug. Comparable pharmacologic evaluation of the emulsifiers is also being undertaken.

Major Findings:

A suspension of fat emulsion is able to solubilize Methyl CCNU and carry it in aqueous solution. The drug possesses the characteristics of a lipid soluble substance following intravenous administration. Comparing the chloroethyl and methyl-cyclohexyl labeled moieties, the following differences

Major Findings cont.

were found. The fast and slow components of the plasma clearance half-life are the same for both labels. Plasma protein binding was 41% ± 3% for chloroethyl and 63% ± 3% of the methylcyclohexyl. The CSF concentration for the chloroethyl is twice that of the ring on the interval 15 minutes to 3 hours. Twice as much of the ring labelled dose is cleared in the urine at 4 hours. The tissue levels of the ring are twice as high as the chloroethyl with the exception of liver, where the ration is reversed. The ether extraction curves correlate well with the rapid component of label clearance.

Significance to Biomedical Research and Program of the Institute:

BCNU is the only nitrosourea which can be delivered intravenously due to its lipid solubility and aqueous insolubility. Pharmacologic evaluation and development of systems to deliver these substances intravenously is of paramount importance to effective cancer chemotherapy.

Proposed Course:

The project awaits the carbonyl label for completion, but results of the chloroethyl and ring label data are being prepared for publication.

Honors and Award: NONE

Publications: NONE

Serial No.: NCI-6282
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Brain Tumor Study Group

Previous Serial Number: NONE

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Mathilde Solowey, Ph.D. and Members of the BTSG

Cooperating Units: Members of the BTSG

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description:

Objectives:

The Brain Tumor Study Group was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of brain tumors utilizing Phase III type studies. More recently, is undertaking Phase II studies in order to make the selection of agents more meaningful.

Methods Employed:

Members of the Brain Tumor Study Group have established a cooperative, ongoing continuous effort providing newly-operated upon patients for randomization to protocol studies. The current protocol is evaluating BCNU and Irradiation Alone and in Combination in a prospective, controlled and randomized study. Administrative support is provided by the Central Office, and institutional support by the contract mechanism. Meetings of the entire Group, at quarterly intervals, provide appropriate communications and exchange of information.

Major Findings:

The current protocol has had approximately 240 patients randomized to its four arms. Preliminary evaluation tends to indicate that BCNU and Irradiation are superior modes of therapy compared to untreated patients. Detailed

Major Findings (con't.):

analysis of these studies is being undertaken.

Significance to Bio-medical Research and Program of the Institute:

These studies are the only prospective, controlled, randomized studies performed in the United States in the treatment of brain tumor. They provide specific detailed and timely information concerning the efficacy of drug therapy for malignancies of the central nervous system.

Proposed Course:

This study will be completed within the next several months. A new protocol evaluating Methyl CCNU and Irradiation will be undertaken. Phase II studies utilizing procarbazine, streptozotocin, dibromodulcitol and adriamycin are being undertaken.

Honors and Awards:

Publications: NONE

Presentations:

"An Evaluation of 1-3-Bis(2-Chloroethyl)-1-Nitrosourea (BCNU) and Irradiation Alone and in Combination for the Treatment of Malignant Glioma."
American Association for Cancer Research Meeting, May 5, 1972, Boston.

Serial No.: NCI-6283
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: An Evaluation of Epipodophyllotoxin (PTG) in a Phase II Study Treating Central Nervous System Neoplasm

Previous Serial Number: NONE

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Mark L. Rosenblum, M.D., Arden F. Reynolds, Jr., M.D. Richard Kaplan, M.D., and Donald Sklansky, M.D.

Cooperating Units: Medical Service, BCRC.

Man Years:

Total:	.5
Professional:	.5
Other:	0.0

Project Description:

Objectives:

This Phase II study is designed to evaluate the efficacy of PTG in the treatment of malignancies within the central nervous system and provide further toxicologic information concerning multiple doses of this lipid soluble drug.

Methods Employed:

Patients harboring intra-cranial malignancy are treated with PTG 100 mg/m^2 per week X eight weeks. Evaluation of neurologic parameters, change in brain scan size and other methods of measuring efficacy are employed. Frequent hematologic and bio-chemical parameters are obtained.

Major Findings:

Twenty-three patients have been treated with epipodophyllotoxin in one or more courses. Because of the mild toxicity encountered, the dose has been increased to 130 mg/m^2 per week X eight weeks. Significant remission of signs and symptoms have been seen in only a few patients and toxicity has been primarily mild leukopenia with some thrombocytopenia and progressive anemia. Although the responses are interesting, they are not of the expected

Major Findings (con't):

order of magnitude.

Significance to Bio-medical Research and Program of the Institute:

Analysis of preliminary results indicates PTG to be less effective than other drugs in the treatment of malignant brain tumor. Significantly higher doses than heretofore proposed can be tolerated.

Proposed Course:

The protocol patient requirement will be completed, the study terminated and the data analyzed in preparation for a manuscript.

Honors and Awards: NONE

Publications: NONE

Serial No.: NCI-6284

1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Methyl CCNU Diffusion from Silicone Capsules: In Vitro and in Vivo.

Previous Serial Number:

Principal Investigator: Mark L. Rosenblum, M.D.

Other Investigators: Donald M Bowie, B.S.
Michael D. Walker, M.D.

Cooperating Units: NONE

Man Years:

Total:	.8
Professional:	.7
Other:	.1

Project Description:

Objectives:

This project is designed to determine the diffusion of 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, (NSC#95441), Methyl CCNU, from silicone capsules in vitro and in vivo, and explore the influence of factors affecting its dynamics.

Methods Employed:

Silicone (dimethylpolysiloxane) capsules are filled with dry crystaline Methyl CCNU to which has been added ^{14}C-chlorethyl label. In vitro studies were performed by suspending the capsules into various fluids, shaking at 37° and sampling at appropriate intervals. The amount of drug diffused is measured by standard isotopic techniques. Capsules were implanted subcutaneously into rats for the in vitro study and the amount remaining after various times analyzed.

Major Findings:

A constant release of Methyl CCNU into the surrounding bath was observed. The diffusion rate decrease over time both in vitro and in vivo, and is proportional to surface area.

Significance to Biomedical Research:

Constant administration of certain chemotherapeutic agents may be advantageous, but at present requires constant IV infusion for constant blood levels. The hazards of long term treatment by that mode is well known. In addition, very hydrophobic agents may not be given IV at all. Subcutaneous insertion of drug filled silicone capsules may provide a constant diffusion of many agents with minimal hazard.

Proposed Course:

The parameters affecting Methyl CCNU diffusion will be further investigated. An in vivo study of this mode of therapy may be undertaken with a rat brain tumor model.

Honors and Awards: NONE

Publications: NONE

Serial No.: NCI-6285
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effect of Polyamines on Rat Brain Nucleic Acid Metabolism

Previous Serial Number: NONE

Principal Investigator: Arden F. Reynolds, Jr., M.D.

Other Investigators: Dianne H. Russell, Ph.D.

Cooperating Units: Section of Enzymology

Man Years:

Total:	.5
Professional:	.5
Other:	0.0

Project Description:

Objectives:

To study the effects of putrescine spermidine and spermine on rat nucleic acid metabolism.

Methods Employed:

Rats are injected intracisternally with a polyamine, then re-injected at a later time with tritiated thymidine or uridine. The method of Fujiolea et al, JBC 238, 3401 (1963) is used for measuring uridine or thymidine uptake in rat brain.

Major Findings:

Preliminary results show a possible stimulatory effect of polyamines and further work is necessary to define the appropriate dosage and timing.

Proposed Course: Continue to define dosage and timing requirements for a statistically significant result.

Honors and Awards: None

Publications: None

Serial No: NCI-6286
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Methyl CCNU: Phase I Toxicologic and Neuropharmacologic Evaluation

Previous Serial Number: None

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Arden F. Reynolds, Jr. M.D. (USPHSH)
Richard Kaplan, M.D.
Donald Sklansky, M.D.
Donald L. Bowie, B.S.
Robert C. Young, M.D.

Cooperating Units: Medical Service, NCI and BCRC

Man Years:

Total:	1.0
Professional:	.8
Others:	.2

Project Description:

Objectives:

This study combines a Phase I human toxicologic evaluation and pharmacologic investigation of this latest nitrosourea. It is designed to determine the maximally tolerated oral dose of MeCCNU, and its pharmacologic characteristics of absorption, distribution, excretion and metabolism.

Methods Employed:

Patients found refractory to other forms of chemotherapy are entered on the Phase I study in coordination with the Medicine Branch, NCI. Careful monitoring of hematologic and toxicologic parameters is obtained. As acceptability of appropriate dose levels is identified, the dose is escalated according to protocol. Select patients receive, along with their therapeutic dose of MeCCNU, radiolabeled tracer doses for pharmacologic study. Blood, urine, cerebral spinal fluid and tissues are analyzed for radioactivity, drug content and metabolites.

Major Findings:

MeCCNU is tolerated in doses up to 290mg/m^2 in previously untreated patients. Lower doses in patients with compromised bone marrow function are required. Absorption of MeCCNU from the gastrointestinal tract is slow with peak plasma levels being reached at 4 to 8 hours. Only a few per cent of the label is found in the stool and recovery of approximately 3/4 of a given dose is obtained from the urine. About 1/2 of the plasma content is protein bound and only a 1/4 appears in the cerebral spinal fluid. TLC of plasma extractions indicates very small amounts of parent compound.

Significance to Biomedical Research and Program of the Institute:

The nitrosoureas are among the most active and antineoplastic compounds available. Determining their tolerable dose, toxicity and pharmacologic characteristics will provide more accurate therapeutic regimens in the future.

Proposed Course:

Patients who continue to be treated at optimal doses of MeCCNU will be evaluated with additional pharmacologic studies to determine tissue content. Emphasis will be placed on the parenteral administration of these substances.

Honors and Awards: NONE

Publications: NONE

Serial No: NCI-6043
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Automated Monitoring of Drug Therapy

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, James K. Hooper

Other Investigators: Michael D. Walker, Richard Hsieh, Barbara Murray, Douglas G. Christian, Peter H. Wiernik

Cooperating Units: Division of Health Services Research, Baltimore USPHS Hospital; Program Analysis Branch, Chemotherapy, NCI, Bethesda, Maryland

Man Years:

Total: 3
Professional: 1
Other: 2

Project Description:

Objectives:

To provide inexpensive, efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

Methods Employed:

An automated method of monitoring drug therapy was designed and implemented.

An inexpensive pen and paper system of processing drug information provided accurate, periodic summaries of patient's drug therapy. A medication for each of a patient's drugs is marked at the time of drug administration. Drug and patient identifiers are typed on the card and this data is read weekly by optical character recognition. The data is collated and a weekly summary is printed listing total dose, frequency of administration or omission, and errors in administration of each drug. A cumulative report printed weekly lists cumulative doses for all drugs the patient has received.

The physician writes his order directly on the medication card that produces four copies: the medicine card copy, the pharmacy copy, and copies for medical records and automated analysis. A clerk types the drug name, dosage

Methods Employed (cont'd)

information, patient name and record number and date drug was initiated. Most intravenous medications can be indicated by "check-off" boxes and each drug administration or drug omission is "checked-off" by the nurse.

Major Findings:

The medication card has proven to fulfill all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by physicians and pharmacists and become a permanent record of drug therapy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded in a concise and orderly fashion in the patient chart.

Significance to Biomedical Research:

This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

Honors and Awards: None

Publications:

Goldman, G., Fortner, C.L., Cradock, J., Murray, B., Hsieh, R., Thompson, W., and Block, J.B.: A Computer Based Medication System for Cancer Chemotherapy Patients: Am. J. Hosp. Pharm., 29, 305-312, (Apr) 1972.

Serial No: NCI-6236
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: Douglas G. Christian, William A. Cornelis, and William R. Grove

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1/8
Professional: 1/8
Other: 0

Project Description:

Objectives:

To evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a medication and allergy history.

Methods Employed:

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart.

Major Findings:

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of

Major Findings (cont'd)

admission-92%, and patients reporting a history of allergy to drugs, food, animals, clothing, and tape-31%. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the hospitalized period. The pharmacists are responsible for placing all known allergic reactions information on the front of the patients drug Cardex, and on the front of the patient chart. At the time of complication of the interview, the original is placed in the history section of the patients hospital chart, and a duplicate is attached to the patients drug profile in the pharmacy. If the pharmacists feels that some of the information should be brought to the physicians attention immediately, he also communicates verbally to the physicians. Two incidences will illustrate this: One patient scheduled to undergo an intravenous Pyelogram mentioned the history of allergy to the pharmacists but not to the physician. Another patient reported to the pharmacist sensitive to general anesthetic. This information was conveyed to the physician for the first time by the pharmacist. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service.

Significance to Biomedical Research:

More complete histories of medication usage are provided which may result in improved care of patients.

Proposed Course:

The present study will be continued.

Honors and Awards: None

Publications:

Cradock, J., Whitfield, G., Menzie, J., and Fortner, C.L.: Post-Admission Drug and Allergy Histories Recorded by a Pharmacist. Amer. J. Hosp. Pharm., 29, 250-252, (Mar) 1972.

Serial No: NCI-6237
1. BCRC
2. Patient Care Pharmacy
4. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of a Patient Care Pharmacy Service

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: Douglas G. Christian, William A. Cornelis, and William R. Grove

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1 1/3
Professional: 1 1/3
Other: 0

Project Description:

Objective:

The pharmacist has traditionally been a product oriented person with little input to the actual care of the patient. The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team activity contributing to the patient's care.

Methods Employed:

The pharmacist makes patient rounds with medical staff. He reviews drug orders and maintains drug profiles on all patients. The profiles are used in the determination of drug-drug interactions, drug-dietary interactions, and drugs that modify laboratory results.

Drug information is provided for the medical and nursing staff by several means; providing verbal or written answers to questions, presenting lectures on pharmacology, and contributing to a drug newsletter.

Major Findings:

The pharmacist has proven that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and in contributing directly to the patient

Major Findings (cont'd)

care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug induced diseases, providing drug information, counselling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities.

Significance to Biomedical Research:

A higher quality of drug prescribing and reduced medication problem has contributed toward better patient care and has enhanced the quality of the research data.

Proposed Course:

Present studies will be continued and additional methods to improve patient care, such as unit dose drug distribution, will be further developed.

Honors and Awards: None

Publications:

Grove, W.R., and Fortner, C.L.: Drug Therapy Surveillance by a Pharmacist in a Cancer Research Center. submitted, Amer. J. Hosp. Pharm., (Mar) 1972

Serial No: NCI-6238
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of a Unit Dose Service and the Preparation of Parenteral Drugs by a Patient Care Pharmacy Service

Previous Serial Number: Same and 6269

Principal Investigator: Clarence L. Fortner

Other Investigators: Douglas G. Christian, William A. Cornelis, and William R. Grove

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 2 1/2
Professional: 2
Other: 1/2

Project Description:

Objectives:

To improving patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

Methods Employed:

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labelled with a name, strength, and controlled number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. All of these parenteral products are prepared aseptically in a laminar air flow environment, and are dispensed in a clearly labelled ready to use form. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particular matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patients name. This information is also recorded by drug and lot number for each investigational agent.

Major Findings:

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduces the possibility of contamination of the drug, permits nurses' to use time more effectively in direct patient care and in general allow the hospital to demonstrate adequate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record.

During the past year the pharmacy service has dispensed approximately 186,964 oral doses of medication to inpatients, 16,876 I.V. admixtures, and 4,456 doses of parenteral investigational cancer chemotherapeutic agents.

Significance to Biomedical Research:

By reducing medication errors, and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients has increased.

Honors and Awards: None

Publications:

Menzie, J., Cradock, J., and Fortner, C.L.: Development of a Unit Dose System for the Handling of Parenteral Investigational Cancer Chemotherapeutic Agents. Amer. J. Hosp. Pharm., 28: 605-610, (Aug) 1971

Serial No. NCI 6023
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Radiation Therapy Service

Principal Investigator: Kirkland C. Brace, M.D.

Other Investigator: Irwin Zarembok, M.D.

Cooperating Units: Baltimore Cancer Research Center and the U.S. Public Health Service Hospital, Baltimore, Maryland.

Man Years: (July 1, 1971 - June 30, 1972)

Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To provide the best possible radiation therapy for the patients of the Baltimore Cancer Research Center and the U.S. Public Health Service Hospital. These facilities are here to provide treatment for the cancer patients on the study program and to provide service radiation therapy for the beneficiaries of the United States Public Health Service.

The majority of the treatments for the Baltimore Cancer Research Center were in connection with other protocols from other Departments. A number of brain tumors were irradiated as part of the Brain Tumor Study. A number of leukemic patients have received irradiation to the brain and spinal cord as part of their total treatment. All patients with pain related to tumor invasion of the bone were treated for palliation. There are a number of other programs in the general hospital which require pre-operative irradiation, particularly for the tumors of the head and neck. The research activities consistute almost eighty percent of the total patient load in this Department.

Methods Employed:

All modes of radiation therapy are available here for the treatment of all types of malignancies. The equipment consists of a 100 Kv superficial therapy machine, a 285 orthovoltage machine, a 5000 curie Cobalt teletherapy unit and will soon be equipped with a 6 MEV linear accelerator. A supply of Cesium, Radium and Strontium sources are available for interstitial and intracavitary radiation therapy.

Major Findings:

During the year ending March 31, 1972, 252 patients were seen in consultation. A total of 211 patients were treated in this Department of which 134 were from the Baltimore Cancer Research Center and the balance from the general hospital. The breakdown of the treatments follow (April 1, 1971 - March 31, 1972):

	Tumor Service	Hospital Service
Superficial Therapy Treatments	0	47
Orthovoltage Therapy Treatments	5	27
Cobalt Therapy Treatment	2819	1103
Intracavitary or Interstitial Radium & Cesium Applications	0	3
Strontium-90 Applications	0	0

Significance:

There has been a general decrease in the hospital population during the past year because of the general curtailment of funds throughout the Public Health Service. The Baltimore Cancer Research Center has had increasing activity during the past year with the result being that the number of patients treated has not changed markedly. The increasing activity in the Hodgkin's Disease Program has been principally responsible for the increase.

Course:

The Radiation Therapy Service will continue to provide treatment for cancer patients both on the Research Service and for the general hospital. The difficulty in procuring and holding personnel continues to provide problems for the maintainence of this Department. The acquisition of a modern accelerator should improve our ability to provide improved treatment for the cancer patients.

Honors and Awards:

None

Publications:

None

Serial No. NCI 6207
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Radiological Physics - A Special Service Which is Supplied for Research Purposes

Principal Investigator: Kirkland C. Brace, M.D.

Other Investigator: Irwin Zarembok, M.D.

Man Years: (July 1, 1971 through June 30, 1972)

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Objectives:

To apply the principles of Radiological Physics to the treatment of patients.

To provide non-patient service irradiations.

To provide a Radiation Safety Program for the Pharmacology Laboratories and Radiotherapy Department.

Methods Employed:

The three Radiation Therapy machines are calibrated periodically. Treatment plans are devised as needed.

Invitro and animal irradiation was provided to investigators in the Pharmacology Laboratory for a total of 115 service irradiations. Irradiation service has been provided for Johns Hopkins, Laboratory of Biology for 80 rabbits and guinea pigs.

A Radiation Safety Program is maintained for the Pharmacology Laboratories in which radionuclides are kept or used. These laboratories are surveyed periodically for radionuclide contamination. The Radiation Therapy facilities (Cobalt-60 Teletherapy unit and various sealed sources of Radium, Cesium-137 and Strontium-90) are surveyed according to AEC Regulations.

Major Findings:

There has been no detectable leakage of any Radiotherapy sources, nor any significant amount of contamination in any Pharmacology Laboratory.

Proposed Course:

It is hoped a new Physicist will be hired during the next few months to reactivate the program and place our surveys on a current basis.

Honors & Awards:

None

Publications:

None

Serial No: NCI 6270
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Combination Chemotherapy and Radiotherapy in the Treatment of Hodgkin's Disease

Principal Investigator: Kirkland C. Brace, M.D.

Other Investigators: Irwin Zarembok, M.D. and Peter H. Wiernik, M.D.

Cooperating Unit: Medicine Service, Baltimore Cancer Research Center

Man Years (July 1, 1971 - June 30, 1972)

Total: 1-1/4

Professional: 1/2

Others: 3/4

Project Description:

Objective:

To determine what role chemotherapy may have in the treatment of early Hodgkin's disease. The role of radiation therapy in the treatment of this disease is now well-established in the early staged disease. The role of chemotherapy in the treatment of the advanced disease is also well-established. In this study we hope to determine if a combination of these two may not provide the ideal treatment for Hodgkin's disease.

Methods Employed:

Three groups of Stage I and II Hodgkin's disease were obtained by randomization of newly diagnosed cases referred to this institution. One group received conventional radiation therapy to the involved areas and adjacent lymph node-bearing areas. A second group of patients were treated with three courses of a combination chemotherapy MOPP and this was followed by irradiation of the involved lymph-node bearing areas. The third group of patients received irradiation to the involved lymph node areas followed by three courses of the combination chemotherapy.

Major Findings:

A total of twenty-one patients randomized into this study have now been followed for not less than two years. There have been recurrences in both the radiation only group and in those patients receiving chemotherapy prior to the radiation. Those patients receiving the radiation therapy followed by chemotherapy have not shown any recurrences as of this date.

Significance:

This study has already clearly shown that the use of the combination chemotherapy in the treatment of early Hodgkin's disease does not effect the curability of the disease, in spite of the fact that it has produced complete tumor regression before the start of the radiation therapy. The data shows that there is a statistically significant difference between those patients receiving the chemotherapy following irradiation and the recurrence rate in the two other groups. This small series suggests that this technique may provide a significantly better cure rate than is possible with the radiation alone.

Course:

The finding that the chemotherapy before radiation therapy does not improve the recurrence rate has resulted in the dropping of this group from the balance of the study. All Hodgkin's disease patients except Stage III-B and IV's will now be randomized to irradiation of the tumor bearing areas and adjacent node-bearing areas or to irradiation of the tumor bearing areas followed by three to six courses of the combination chemotherapy. This should provide significant data in the next several years to fully establish the role of this combination in the treatment of Hodgkin's disease.

Honors and Awards:

None

Publications:

None

Serial No: NCI 6294
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Total Body Irradiation in the Treatment of Advanced Lymphosarcoma

Principal Investigator: Kirkland C. Brace

Other Investigator: Irwin Zarembok, M.D.

Cooperating Units: Baltimore Cancer Research Center, Medicine Service

Man Years: (July 1, 1971 - June 30, 1972)

Total: 1/8
Professional: 1/16
Other: 1/16

Project Description:

Objectives:

To study the use of whole body irradiation in the treatment of lymphosarcoma, Stages III and IV.

Methods Employed:

Eleven patients with advanced lymphosarcoma were treated with whole body irradiation, either alone or in combination with more localized irradiation. Doses employed were usually ten to twenty rads per week until significant depression of the white blood count or platelet count occurred.

Major Findings:

Although many patients achieve either partial or complete remission, these remissions were short-lived. All patients but one relapsed within one year following completion of treatment. When attempts were made to treat these patients with myelosuppressive chemotherapeutic agents, bone marrow tolerance was found to be poor.

Significance:

Whole body irradiation was employed with good initial results with almost no major short-term morbidity. However, the brief duration of remissions and the poor marrow tolerance to chemotherapy make it unlikely that this method of treatment will have better results than more conventional regimens.

Proposed Course:

We will continue to follow these patients in order to obtain more long-term data on the quality and duration of their survival.

Honors & Awards:

None

Publications:

None

SUMMARY REPORT

LABORATORY OF PHARMACOLOGY

Continuing studies on the anthracycline antibiotics have resulted in a greater understanding of the pharmacology of these drugs. The development of a fluorescent assay, for example, has helped elucidate the enzymatic pathway from daunomycin to the pharmacologically active compound in man. The enzymatic alteration in structure brought about in the anthracycline moiety of the antibiotic was shown to profoundly affect the retention of the drug by the cell. An understanding of this metabolism resulted, in turn, in the development of a single dose drug regimen which significantly reduced toxic effects, yet increased the therapeutic efficacy of the drugs.

The development of a general assay utilizing the presence of polyamines in the urine of patients with diagnosed cancer has been described. These substances shown to be present in the urine of cancer patients at considerably higher concentrations than in the urine of non-cancer patients may prove to be of inestimable value, not only in detecting early cancer, but modulating chemotherapy in response to the patient's diseased state. At present, studies attempting to relate these polyamines to the amount of tumor tissue present are under active investigation. In general, too, a more fundamental understanding of the role polyamines play in cellular metabolism has been intensely examined. In rapidly proliferating tissues it has been shown that there is a good correlation between the increased concentration of these substances and the increased synthesis of ribosomal RNA. Further, these polyamines can cause structural alterations in the nucleolus of liver tissue, which can in turn alter profoundly RNA polymerase activity. Studies have also indicated that RNAase activity can be increased several fold in the presence of putrescine and spermidine. Specific RNAases have been prepared from microbial sources in an attempt to develop means by which the RNA from an oncogenic virus may be sequenced. Thus far a cytidylate, a uridylate and an adenylate, specific enzymes have been described. Attempts are under way, at present, to isolate an homogeneous strand of RNA from an oncogenic virus. After the conclusive demonstration of the inhibition of an RNAase from whatever source by polyguanylic acid, this substance, or a lower molecular weight version of it, is being examined for its potential antibiotic properties.

Immunologic research has been concentrated in in vitro systems designed both to detect host response to specific antigens as well as the amplification of responses to tumor specific antigens. The use of the synthetic polynucleotide complex-polyadenylic-polyuridylic acid has played an important role in increasing the sensitivity of the host response to specific antigens. This amplification will allow for discerning the presence of antigens at levels several fold lower than previously measured. In addition, clinical quantification of host immune function during illness and during chemotherapy has been advanced by improvements in the techniques of frozen cell usage. Characterization of tumor specific antigens has been brought about by the use of blocking antibody in inhibiting antibody formation to antigens common to both normal and neoplastic cells. This approach aborts antigenic competition and allows for the recognition of antigens heretofore unrecognized. In addition, immune complex disease has been shown to be present in patients with neoplasia and in two cases, now studied, the antigen in this complex has been shown to be

related to oncogenic RNA viruses. Similarly, immune complex disease in AKR mice have been shown to consist of gs-1 and gs-3 antigens of the Gross virus. Finally, studies concerning the immunologic effects of BCG in man have shown that BCG primarily enhances the response to antigens to which the host has been exposed previously, i.e. the secondary immune response. The clinical cell program has pointed out some of the problems involved in the utilization of platelet products stored at 25° C.

Serial No. NCI-6012
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6012

Principal Investigator: Nicholas R. Bachur

Other Investigators: Malcolm Gee

Cooperating Units: Laboratory of Chemistry, National Heart & Lung Institute
Drug Development Branch, NCI, NIH

Man Years (July 1, 1971 to June 30, 1972)

Total:	1.4
Professional:	0.4
Other:	1.0

Project Description:

Objectives:

To study the biochemical pharmacology of selected cancer chemotherapeutic agents to provide a scientific foundation for their usage. To resolve the metabolic pathways of cancer chemotherapeutic agents and to study the interactions of these drugs and their metabolites with tissue components in vitro and with organized physiological systems in vivo.

Major Findings:

We have previously shown that the anthracycline antibiotics, daunorubicin and adriamycin, are extensively metabolized both in vivo and in vitro by mammalian systems. We have attempted to define and to isolate each of the biochemical reactions in which the anthracycline antibiotics participate. One group of reactions which we studied have been those occurring within microsomal fractions. Adriamycin, daunorubicin, and the metabolite, daunorubicinol, undergo a reductive cleavage of their glycosidic bond in the presence of rat liver microsomes to form their respective deoxyaglycone products. Analysis of the products by mass spectroscopy, nuclear magnetic resonance and infrared spectroscopy verified the deoxy structures. Rat brain and kidney homogenate also are active in the catalysis of this reaction. Molecular oxygen reversibly inhibits both the microsomal and a soluble

preparation of this enzyme. Both the microsomal and soluble preparation require NADPH for activity.

This enzyme, which is a reductive glycosidase, has been solubilized and partially purified from the rat liver microsomes by protein purification techniques. The importance of this enzyme in the metabolism of the drugs in humans is being evaluated. When animals are placed in reduced oxygen tension atmospheres, there occurs an increase of the anthracycline glycosidase activity resulting in the in situ formation of aglycones. What the relationship of the aglycone formation is to toxicity or drug efficacy is unknown.

The purification of our previously described enzyme, daunorubicin reductase which has been found in all mammalian tissues tested, has been partially accomplished. Through the use of protein isolation techniques, we have obtained an enzyme from rat liver which is several hundred fold purified. We are comparing the characteristics of the rat liver enzyme with enzyme from human blood erythrocytes. Since this enzyme appears to be of some importance in the pharmacodynamics of daunorubicin activity, we are comparing enzyme from various sources and attempting to relate it to the actions of the drug.

Significance to Biomedical Research and Program of the Institute:

The understanding of the biochemical alterations that active chemotherapeutic agents undergo is central to the understanding of their usage. This is of importance with the anthracycline antibiotics since we have observed a rather extensive metabolism of both adriamycin and daunorubicin in vivo. Although these drugs have been used for several years, their metabolism has not been described.

Proposed Course:

We intend to further delineate the metabolic pathway of adriamycin and daunorubicin and to study the interaction of these drugs and their metabolites with the enzymes and various subcellular organelles of both sensitive and resistant tumors as well as with normal tissues.

Honors and Awards: None

Publications:

Bachur, N.R. and Huffman, D.H.: Daunorubicin Metabolism: Estimation of Daunorubicin Reductase. Brit. J. of Pharmac. 43: 828-833, 1971.

Serial No. NCI-6014
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Cellular Control Mechanisms Affecting Cell Growth and Differentiation

Previuos Serial Number: NCI-6014

Principal Investigators: Nicholas R. Bachur
Douglas R. LaBrecque

Other Investigators: Marta Kaber

Cooperating Units: None

Man Years (July 1, 1971 to June 30, 1972)

Total:	2.2
Professional:	1.2
Other:	1.0

Project Description:

Objectives:

To study the control mechanisms which affect cellular growth and differentiation. These studies are aimed at elucidating control principles effecting all levels of cellular machinery as well as various levels of physiological control of the organized functioning animal. To determine difference in controls between differentiated and nondifferentiated tissues and between malignant and normal tissues. To study the effects of control changes on physiologic processes.

Major Findings:

As a continuation of our studies on the materials which promote blastogenesis in nonproliferating lymphocytes, we have purified a specific leukoagglutinin from phytohemagglutinin by protein purification techniques. This material has several activities on cellular physiology. It promotes agglutination specifically of leukocytes and stimulates leukocyte transport of several materials. Also, the material is a potent mutogen which produces over 95% stimulation of nonproliferating lymphocytes to blastogenesis. The chemical characteristics and mechanisms by which these cellular changes occur are being investigated.

Studies on soluble dihydrofolate reductase from methotrexate resistant choriocarcinoma of human placenta and testes. Lung metastases from choriocarcinoma were compared to determine whether there was a biochemical explanation for their resistance to methotrexate therapy. The sensitivity of dihydrofolate reductase to methotrexate inhibition was nearly identical in normal term placenta, placental choriocarcinoma, and testicular choriocarcinoma, $I_{50} \times 10^{9}$ M of 8.6, 9.3, and 9.3, respectively. There was no appreciable peptidase G activity in either the normal or malignant tissues. The dihydrofolate reductase activity of normal placental tissue was higher than that found in either case of choriocarcinoma but several interpretations of these data are possible.

We have detected the presence of and partially purified a material which is present in the liver of rats undergoing hepatic regenerations that will stimulate nucleic acid synthesis in tissue preparations from recipient rats.

Significance to Biomedical Research and Program of the Institute:

The isolation of materials from both plant and mammalian sources which effect the growth and differentiation of normal lymphocytes and hepatocytes is paramount in the study of the processes which control cellular activities. By using these purified materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways is possible, we are approaching techniques where chemotherapeutic agents may be used to control cellular growth, differentiation, and proliferation. This is, of course, classically demonstrated in the use of methotrexate to inhibit dihydrofolate reductase.

Proposed Course:

We intend to continue our studies on the mechanism of action of the leukoagglutinin and the mammalian promotor substance.

Our studies on dihydrofolate reductase have been terminated.

Honors and Awards: None

Publications:

Jarabak, J. and Bachur, N.R.: Biochemical Studies of Methotrexate Resistance in Choriocarcinoma of the Human Placenta and Testis. Biochim. Med. 5: 430-435, 1971.

Serial No. NCI-6016
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Pharmacodynamics of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6016

Principal Investigator: Nicholas R. Bachur

Other Investigators: Richard Adamson
Robert Pitillo

Cooperating Units: Laboratory of Chemical Pharmacology, NCI, NIH
Southern Research Institute, Birmingham, Alabama

Man Years (July 1, 1971 to June 30, 1972)

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives:

To investigate the biochemical and physiological effects of anticancer drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Major Findings:

Comparative studies of adriamycin and daunorubicin were carried out with L1210 tumor cells grown in spinner-flask tissue culture. The effects of daunorubicin and adriamycin on nucleic acid metabolism were studied in vitro with labeled nucleoside precursors and compared with the established effects of actinomycin D. L1210 ascites tumor cells incubated with daunorubicin under physiological conditions of pH, temperature, and tonicity showed significantly greater inhibition of tritiated thymidine incorporation into DNA and carbon 14-labeled uridine incorporation into RNA than cells treated with equimolar concentrations of adriamycin. Studies of uptake of daunorubicin and adriamycin by L1210 cells in vitro showed significant differences. At 37°C the uptake of daunorubicin was more than twice that of adriamycin. The uptake of both anthracycline drugs was temperature sensitive which suggests an energy dependence for drug uptake rather than simple diffusion into cells. However,

a portion of the observed drug uptake may be due to binding independent of temperature since at 4°C about 6% of the adriamycin and 15% of the daunorubicin was associated with the L1210 cells. Daunorubicin, a slightly less polar compound than adriamycin, could bind to membranes more readily and account for the comparative increased binding.

Another important difference between these drugs was that daunorubicin was metabolized to daunorubicinol by the L1210 cells while no similar metabolite of adriamycin could be demonstrated. Similar observations in other in vivo and in vitro experiments had been made in our laboratory. No aglycone formation was seen in these experiments. Other animal tissues have glycosidase activity and can cleave the anthracycline antibiotics but the intact L1210 cells apparently do not. When the uptake data and inhibition data were compared, daunorubicin was apparently a more potent inhibitor of nucleic acid synthesis than adriamycin but the cell-bound concentration of daunorubicin is much greater at 37°C than that of adriamycin. When these results were calculated, they indicated that the actual inhibitory effectiveness of adriamycin is as great or greater than daunorubicin but that the cellular uptake of adriamycin is slower.

When equimolar levels of daunorubicin and daunorubicinol were compared, the daunorubicin was a more effective inhibitor of DNA synthesis than was daunorubicinol by a factor of from 2.6 to 3.5 times. However, daunorubicin at 1 μM was 19 times more effective than daunorubicinol for inhibiting RNA synthesis. These differences indicated basic differences in the mode of action.

The studies on the uptake of daunorubicin and daunorubicinol by L1210 cells showed significant differences. At 37°C the uptake of daunorubicin was 2.7 times greater than that of daunorubicinol. It is interesting that the drug uptake of 2.7 times is very near to the difference seen in inhibition of thymidine incorporation range of 2.6 to 3.5 times. Assuming that the drug associated with L1210 cells is all biochemically active, it appears that daunorubicin and daunorubicnol have about equal effects on the incorporation of tritiated thymidine into DNA but have different effects on the incorporation of uridine into RNA. No aglycone formation was observed in these experiments with intact L1210 cells. None of the anthracycline antibiotics effect the transport of the nucleic acid precursors into the L1210 cells, and the inhibition appears to be solely at the synthesis level of polymerized nucleic acid.

Significance to Biomedical Research and Program of the Institute:

It has been shown that the therapeutic spectrum as well as the therapeutic indices of daunorubicin and adriamycin are different. Since the structures of these two antibiotics are very similar this is of some importance.

Of equal importance is the demonstrated inhibitory activity of the metabolite, daunorubicinol. Although this metabolite is similar in structure to adriamycin, it appears to possess different activity. An examination of these differences may provide us with insight into the mechanism of action of the anthracyclines and may allow for the synthesis of better antitumor agents.

Proposed Course:

Through the continued investigation of the pharmacodynamics of these drugs, we may be able to determine why the drugs possess such differences in the therapeutic index and spectrum of action.

Honors and Awards: None

Publications:

Meriwether, W.D. and Bachur, N.R.: Inhibition of DNA and RNA Metabolism by Daunorubicin and Adriamycin in L1210 Mouse Leukemia. Cancer Research, June, 1972.

Serial No. NCI-6241
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Clinical Pharmacology of Cancer Chemotherapeutic Agents

Previous Serial Number: NCI-6241

Principal Investigator: Nicholas R. Bachur

Other Investigators: Robert S. Benjamin
William H. Greene
Peter H. Wiernik

Cooperating Units: Medicine Service, Baltimore Cancer Research Center, NCI

Man Years (July 1, 1971 to June 30, 1972)

Total:	1.4
Professional:	1.4
Other:	0.0

Project Description:

Objectives:

To study the disposition of cancer chemotherapeutic agents in humans with respect to absorption, distribution, biotransformation, and excretion. To characterize drug effects, potency, and maximum efficacy. To evaluate the toxicity of cancer chemotherapeutic agents in man and observe various aspects of biological variations and other factors such as age, body weight, sex, route of administration, time of administration, rate of inactivation and excretion, tolerance, pathological state, genetic factors, and drug-drug interaction. To devise appropriate dosage schedules. To develop ancillary therapy to minimize toxic effects of drugs without effecting the therapeutic value.

Major Findings:

Daunorubicin metabolism was examined *in vitro* by using samples of human peripheral, acute myelogenous leukemic cells. These cells contained the enzyme daunorubicin reductase, a cytoplasmic enzyme that converts daunorubicin to its principal metabolite, daunorubicinol. The enzymes from both normal and leukemic leukocytes require NADPH for activity and have similar kinetics and substrate saturation characteristics with a Km of 1.7×10^{-4} M for daunorubicin

When daunorubicin reductase levels in leukemic myeloblasts were related to clinical response to daunorubicin therapy, we found that the patients with a high enzyme level responded favorably to daunorubicin therapy with either a complete or partial remission whereas those patients with a lower enzyme level experienced either no response or died during therapy. These observations suggested that the level of daunorubicin reductase in the leukemic myeloblasts may be important in determining the susceptability of that cell to daunorubicin. Moreover, the measurement of daunorubicin reductase level may have prognostic value regarding the clinical response to daunorubicin therapy. When homogenates of leukemic myeloblasts were analyzed for daunorubicin metabolism, the only fluorescent metabolite found was daunorubicinol. This was similar to what we observed with normal blood components. Since no interfering reactions were present, we used the myeloblast homogenate to study the daunorubicin reductase reaction without purification of the enzyme. The enzyme in both normal human blood homogenates and myeloblast homogenates utilize NADPH as a cofactor. NADH has about 15% of the activity of NADPH. Both NAD and NADP were inactive. There was no relationship of leukocyte daunorubicin reductase levels to sex. However, when an attempt was made to evaluate the effect of patient's age on daunorubicin reductase levels, a regression analysis was performed which demonstrated a significant relation between age and reductase level. It appeared, therefore, that poorer response rate for the older group of patients was at least in part related to the tendency of this older group of patients to have lower daunorubicin reductase levels.

Additional studies on the clinical pharmacology of adriamycin have been carried out and are reported in Project #NCI-6279.

Significance to Biomedical Research and Program of the Institute:

The studies on the clinical pharmacology of these anthracycline antibiotics have been the major contributory factors to the development of new dosage regimens as well as prospective studies on ancillary therapeutic measures to reduce toxicity and increase efficacy of these drugs.

Proposed Course:

The project is to be continued and expanded.

Honors and Awards: None.

Publications:

Huffman, D.H. and Bachur, N.R.: Daunorubicin Metabolism by Human Hematologic Components. Cancer Research. 32: 600-605, 1972.

Huffman, D.H. and Bachur, N.R.: Daunorubicin Metabolism in Acute Myelocytic Leukemia. Blood. June 1972.

Serial No.: NCI-6210
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Development of Specific RNAses as an Aid in the Sequencing of t-RNAs from Murine Leukemia Viruses

Principal Investigator: C. C. Levy

Other Investigator: M. Schmukler

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives
The isolation from microbial sources of nucleases which will hydrolyze nucleic acids at specific linkages as an aid in the sequential analysis of t-RNA from various tumor or viral sources.

Major Findings
The technique of enrichment culture was employed to isolate a number of microorganisms from soil which could degrade RNA at specific nucleotide bonds. Thus far, nucleases specific for cytidylic acid and uridylic acid internucleotide bonds have been obtained from two of the organisms. A third, Serratia sp., elaborates an enzyme which shows preferential specificity for adenylic acid linkages.

A number of preliminary attempts to isolate homogeneous t-RNA or other forms of RNA from various murine leukemia viruses have had limited success, in that only small amounts of virus material was available. However, the material thus far obtained is about 80% homogeneous as judged by polyacrylamide gels. Currently under study is the most efficient means of obtaining larger amounts of virus.

Significance to Cancer Research
Nucleic acid metabolism or, more correctly, the alteration of nucleic acid metabolism is believed to play an important role in cancer induction. It is hoped that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer from tumor cells may provide a clue as to the nature of cancer induction.

Projected Course

To isolate homogeneous t-RNA from viral sources and to attempt a sequential analysis of its nucleotide composition.

Publications

Levy, C. C. The properties of a ribonuclease from Serratia sp. with a proclivity for adenylic acid residues. Biochim. Biophys. Acta 246, 464-475 (1971).

Levy, C. C. Enterobacter ribonuclease. II. A reexamination of the enzyme's specificity toward internucleotide linkages. Biochim. Biophys. Acta 246, 476-486 (1971).

Mitch, W. E., Jr. and Levy, C. C. Anomalous behavior of ribonuclease A on Sephadex G-100. Biochim. Biophys. Acta 251, 388-392 (1971).

Mitch, W. E., Jr. and Levy, C. C. Residue specificity of an endonuclease isolated from Aerobacter sp. Biochim. Biophys. Acta 259, 69-75 (1972).

Friedling, S. P., Schmukler, M., and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. I. Purification and general properties. Biochim. Biophys. Acta, in press.

Schmukler, M., Friedling, S. P., and Levy, C. C. Polyguanylic acid-inhibited ribonuclease of Klebsiella. II. Studies with synthetic polyribonucleotides. Biochim. Biophys. Acta, in press.

Serial No. NCI-6242
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Polyamines and Cancer: Functional Significance

Principal Investigator: D. H. Russell

Other Investigators: C. C. Levy

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives
To define the functions of putrescine, spermidine, and spermine, and to use this information for cancer control.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by homogenization in acid, alkaline butanol extraction and high voltage electrophoresis for separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine; or from ^{14}C-spermine formation from ^{14}C-spermidine.
4. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
1. Utilization of the various methods for detecting polyamines in the urine of cancer patients continues to reaffirm the validity of the initial observation, namely, that these compounds are elevated in patients with cancer.
2. Investigations into the pathways of polyamines synthesis have been undertaken to examine the possible relationships between these compounds and neoplastic growth. These studies have revealed the presence of a possible metabolite of spermidine in the urines of both normal and cancer patients. The concentration of this unknown, as judged by ninhydrin color, is about

10-fold greater in cancer patients. Attempts to identify the compound are in progress. In addition, two inhibitors of the enzyme responsible for putrescine formation (ornithine decarboxylase) have been found. These are α-methyl ornithine and N-5-methyl ornithine. Currently, extensive screening procedures have been undertaken to find the possible effects of these agents on various animal tumors.

3. Considerable data have been accumulated concerning the effects of polyamines on nucleolar RNA polymerase activity. Nucleolar RNA polymerase (RNA polymerase I) was partially purified from rat liver nucleolar preparations and stabilized by the addition of albumin. It was observed that there was a 2-3 fold increment in RNA polymerase I activity when the enzyme was assayed in the presence of spermidine as opposed to that found in the presence of magnesium. In addition to the stimulation of RNA polymerase I activity, the polyamines caused structural alterations in the nucleolus. Electron micrographs showed definitive changes in the nucleoli when the nuclei were isolated in the presence of 2.5-10 mM putrescine, spermidine, or spermine, in contrast to that seen with similar concentrations of magnesium. Nuclei isolated by the addition of either spermidine or spermine as the stabilizing cation had nucleoli that were significantly larger than those isolated with magnesium. In general, when the nuclei were isolated in the presence of magnesium, the nucleoli were compact and electron dense, whereas in the presence of spermidine or spermine, the nucleolonemas were less aggregated, and the nucleoli contained lacunae. Both spermidine and spermine (10 mM) caused the formation of ring-shaped nucleoli.

Significance to Cancer Research

Considerable progress has been made in understanding the functional role that polyamines play in the growth process. The possibility that these compounds may be enhanced, directly or otherwise, in processes leading to abnormal growth is beginning to be realized. Thus, for example, the structural alterations in cellular organelles (cited above) is suggestive that other modifications of structure leading to abnormal growth can occur.

Proposed Course

1. Further studies involving regular screening of AML's before, during and after chemotherapy will be undertaken to determine if polyamine levels at any given point in therapy can serve as a reliable indicator for future chemotherapy.
2. Once the possible metabolite of spermidine isolated from cancer patients can be ascertained, then the enzyme responsible for its formation will be isolated. The enzyme could be useful to lower spermidine levels in tumors and to determine if this, in turn, affects tumor growth.
3. The inhibitors discussed above will be examined for their ability to inhibit tumor growth in various animal systems.
4. Finally, since spermidine affects RNA so profoundly, further studies on this enzyme will be continued, to determine if this enzyme can be used as an indicator of neoplastic growth.

Publications

Russell, D. H.: Increased polyamine concentrations in the urine of human cancer patients. Nature 233: 144-145, 1971

Russell, D. H., Levy, C. C., Schimpff, S. C., and Hawk, I. A.: Urinary polyamines in cancer patients. Cancer Res. 31: 1555-1558, 1971

Russell, D. H., Levy, C. C. and Taylor, R. L.: Increased DNA associated with rat liver nucleoli when isolated with spermidine. Biochem. Biophys. Res. Commun., in press

Russell, D. H., Levy, C. C., Schimpff, S. C., and Hawk, I. A.: Implications of polyamines in the body fluids of cancer patients. J. Natl. Cancer Inst., in press

Serial No. NCI-6244
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH

Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Fatty Acid Synthesis by Human Platelets

Principal Investigators: M. Schmukler
P. Zieve

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives
To study the control of fatty acid synthesis in human platelets and to note similarities to and differences from other tissues.

Major Findings
When washed human platelets are incubated in vitro, glucose stimulates fatty acid synthesis. By use of inhibitors of glycolysis, the monophosphate shunt, and the Krebs cycle, it was concluded that the effect of glucose required the operation of both the shunt and the Embden-Myerhoff pathway.

Significance to Cancer Research
Control of hemorrhage is a major problem in leukemia and in patients receiving cancer chemotherapy. Platelet transfusions are often needed to stop bleeding. The viability of platelets is dependent upon their continued normal metabolism, so that factors involved in the control of platelet metabolism must be delineated in order to preserve platelet viability for transfusions.

Proposed Course
Study completed. Project terminated.

Publications - None

Serial No. NCI-6245
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Human Platelet Carbohydrate Metabolism: (1) The Effect of Cyclic AMP; (2) Interactions of Thrombin and Nucleotides and Their Effects on Glycolysis; (3) Effects of Storage and Various Drugs on Glycogenesis and Glycogenolysis

Principal Investigators: P. Zieve
M. Schmukler

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives

To study the metabolism of platelets in order to define, if possible, the biochemical factors involved in platelet aggregation.

Major Findings

1. Thrombin initiates platelet aggregation and at the same time causes a marked stimulation in platelet lactate production. Cyclic AMP, an inhibitor of aggregation, stimulates conversion of glycogen to glucose in the platelet, while at the same time inhibiting lactate production. It was found that cyclic AMP inhibits glycolysis at the phosphofractokinase step and at the same time activates glycogen phosphorylase.
2. ADP and ATP have no effect upon glycolysis in intact platelets but inhibit stimulation produced by thrombin. On the other hand, thrombin has no effect upon lactogenesis in disrupted platelets but inhibited its stimulation by ATP and ADP. It was found that these nucleotides were able to alter the electrophoretic mobility of thrombin, possibly by binding to the protein.
3. Platelets stored at 4° C and 22° C have been shown to have impaired capability for glycogenesis. Some of this deficit can be revised by addition of uridine to the platelets. Platelets stored with PGE or PGA demonstrate less of a loss of glycogenic capability.

Significance to Cancer Research

Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course

Further studies are planned on the effect of aggregation stimulators and inhibitors on platelet glycolysis. Studies of thrombin-nucleotide interaction and effect of storage on glycogen metabolism will be continued.

Publications

Zieve, P. D. and Schmukler, M. The effect of cyclic AMP on glycogenolysis and glycolysis in human platelets. Biochim. Biophys. Acta 252, 280 (1971).

Serial No. NCI-6246
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Humoral Control of Fatty Acid Synthesis by Human Platelets

Principal Investigators: M. Schmukler
P. Zieve

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives
To study mechanisms involved in the control of fatty acid synthesis in human platelets and to study evidence suggesting the existence of a humoral factor which stimulates lipogenesis.

Major Findings
Platelets isolated from individuals who have been fed glucose incorporate acetate into fatty acids more rapidly than do control platelets. While a similar effect was noted after fructose feeding, glactose feeding had no effect. Preincubation of whole blood with glucose prior to platelet isolation did not stimulate fatty acid synthesis. Glycogen and lactate levels and rates of lactogenesis, glycogenolysis, and glucose oxidation were not affected by glucose feeding.

Significance to Cancer Research
Major causes of death in leukemia are infection and hemorrhage. These are also complications of chemotherapy. The platelet plays a major role in the clotting mechanism and platelet transfusions are used to control hemorrhage in cancer patients. Knowledge of the metabolism of platelets and of factors involved in platelet stability are therefore necessary to allow effective use of these cells in control of bleeding.

Proposed Course
Research completed. Project terminated.

Serial No. NCI-6247
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Purification of Bacterial and Mammalian Nucleases with Differing Residue Specificities

Principal Investigator: M. Schmukler

Other Investigators: C. C. Levy

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives

Purification of a ribonuclease from Klebsiella species, and to delineate the characteristics and properties of human ribonucleases and determine whether any cancer-related changes occur. To purify ribonucleases from plasma and white cells in normal subjects and patients with cancer, and characterize and compare their properties.

Major Findings

1. A ribonuclease from Klebsiella grown on deoxy AMP or DNA as a nitrogen source has been purified 550-fold. It shows a specificity for poly A and poly U, artificial polymers, but is able to digest ribonucleic acid completely, releasing 2'-3' cyclic mononucleotides. The enzyme has no cation requirement and has a pH optimum at 7.0 in phosphate and 7.5 in Tris. Hydrolysis of RNA or poly U by this nuclease is inhibited competitively by poly G, poly I, poly X, poly GU, poly GT, and poly GC. Inhibition of nucleases by this class of compounds has not previously been reported.
2. Leucocyte ribonuclease can hydrolyze native RNA and poly U but not poly C, poly G, or poly A. The optimum pH is 7.0 and activity is greater in Tris-HCl than phosphate buffer. Plasma contains considerable ribonuclease activity. Poly C is hydrolyzed most rapidly in plasma, at a rate four times that for RNA hydrolysis, while poly U is hydrolyzed at 50% of the rate for RNA. Thus far, AML patients show no differences from normal controls.

Significance for Cancer Research

The study of enzymes concerned with nucleic acid metabolism is essential to

an understanding of the mechanisms of carcinogenesis and cancer control since there is obviously an alteration in nucleic acid metabolism in cancer and since most chemotherapeutic agents are directed in some way against nucleic acid metabolism. Nucleases which have some residue specificity should be valuable tools in the delineation of nucleic acid structure. It is also possible that nucleases with some type of specificity may be useful as chemotherapeutic agents. The studies with the polyribonucleotide inhibitors of nuclease activity may be useful in suggesting techniques whereby specific functions of malignant cells could be disrupted.
2. Nucleic acid metabolism is altered in malignancy. Study of enzymes involved in nucleic acid metabolism may offer clues as to the nature of the malignant process. These enzymes, also, may be indicators of certain types of malignancies.

Proposed Course
1. After stabilizing enzyme at its present purity, specificity studies, kinetics, and anticancer properties will be studied. Studies of nuclease inhibition by synthetic polyribonucleotides will be carried on with other purified nuclease such as micrococcal nuclease.
2. Studies will be continued to determine if differences between nucleases isolated from cancer and normal cells can be used as a marker to indicate the presence of disease.

Publications
Friedling, S. P., Schmukler, M., and Levy, C. C.: Polyguanylic acid-inhibited ribonuclease of Klebsiella. I. Purification and general properties. Biochim. Biophys. Acta, in press

Schmukler, M., Friedling, S. P., and Levy, C. C.: Polyguanylic acid-inhibited ribonuclease of Klebsiella. II. Studies with synthetic polyribonucleotides. Biochim. Biophys. Acta, in press

Serial No. NCI-6258
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Glycosidase Levels in Serum, White Cells and Marrow of Leukemic Patients

Principal Investigators: M. Schmukler
J. Holtzman

Other Investigators: None

Cooperating Unit: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	3/8
Professional:	3/8
Other:	0

Project Description:

Objectives
1. To see whether there is a correlation between existence of malignancy and glycosidase levels.
2. To see whether glycosidase levels correlate with presence or absence of drug resistance.
3. To see whether glycosidase levels reflect remission or relapse.

Major Findings
Techniques to the study are being set up. Umbelliferone substrates are being synthesized.

Significance to Cancer Research
Data from this study may aid in the detection of malignancy and may detect the onset of drug resistance, thereby helping the chemotherapist to develop a more rational regimen.

Proposed Course
Preliminary studies were negative. Project terminated.

Serial No. NCI-6287
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Glycosidases of Human Platelets

Previous Serial Numbers: None

Principal Investigators: M. Schmukler
P. D. Zieve

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years:
Total: 1 1/4
Professional: 3/4
Other: 1/2

Project Description:

Objectives
To study platelet glycosidases, their release reaction, and the relationship of these enzymes to platelet function and stability.

Major Findings
Five platelet glycosidases (β-galactosidase, α-mannosidase, N-acetyl-β-glucosaminidase, β-glucusonidase, and N-acetyl-β-galactosaminidase) are released from washed platelets after addition of thrombin. This release can be inhibited by PGE, ATP, CTP, and GTP. The release of glycosidases is decreased after storage, the decrease being greater in platelets stored at 22° C than in those at 4° C. Glucose is able to augment thrombin-induced release in both fresh and stored platelets. There is no damage in platelet glycosidase levels (except for a marked drop in α-mannosidase) in platelets stored 3 days at room temperature, although the plasma levels rise.

Significance to Cancer Research
Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course
Further studies of inhibitors of glycosidase release are in progress. Storage with membrane stabilizers will be attempted to note whether these have a favorable effect on platelet viability and function.

Publications - None.

Serial No. NCI-6288
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Polyamine Biosynthesis in Rapidly Growing Tissues

Principal Investigator: D. H. Russell

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives
To elucidate the pattern of synthesis and accumulation of polyamines in rapidly growing biological systems. The drugs trimethylcholanthrene, 3,4 benzpyrene, clofibrate, phenobarbitol, and heparin were used to study their effects on the polyamine biosynthetic pathway in developing rat liver.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by homogenization in acid, alkaline butanol extraction and high voltage electrophoresis for separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine; or from ^{14}C-spermine formation from ^{14}C-spermidine.
4. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
The most immediate observation was that all the drugs studied produced large elevations of both ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. The alteration in activity of both enzymes in the polyamine pathway was paralleled and followed by increased incorporation of uridine-6-[^3H] into RNA. The close correlation observed between RNA and polyamine synthesis is suggestive of a strong interrelationship between them.

To examine if this is indeed the case autoradiographic studies, using ^{3}H-putrescine and ^{3}H-uridine were conducted to examine the distribution of putrescine during RNA synthesis. The highest accumulation was found in the nucleolus - the organelle where ribosomal RNA is synthesized.

Significance to Cancer Research

An attempt is being made to elucidate the control process in rapidly growing cells. An understanding of this process, it is hoped, will gain understanding of the neoplastic process itself.

Proposed Course

These studies will be continued with particular emphasis on elucidating in considerably greater detail the relationship in the nucleolus between RNA and polyamine synthesis.

Publications

Russell, D. H., and McVicker, T. A.: Polyamine metabolism in mouse liver after partial hepatectomy. Biochim. Biophys. Acta 244: 85-93, 1971

Russell, D. H., and McVicker, T. A.: Polyamines in the developing rat and in supportive tissues. Biochim, Biophys. Acta 259: 247-258, 1972

Russell, D. H., Shiverick, K. T., Hamrell, B. B., and Alpert N. R.: Polyamine synthesis during initial phases of stress-induced cardiac hypertrophy. Amer. J. Physiol. 221: 1287-1291, 1971

Russell, D. H.: Drug stimulation of putrescine and spermidine syntheses. Biochem. Pharmacol. 20: 3481-3491, 1971

Gfeller, E., and Russell, D. H.: Autoradiographic distributions of ^{3}H-putrescine and ^{3}H-uridine in a Xenopus liver cell line. Z. Zellforsch 120: 321-331, 1971.

Serial No. NCI-6289
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Purification of S-adenosyl-L-methionine Decarboxylase from Rat Liver and from other Biological Sources

Principal Investigator: D. H. Russell

Other Investigators: C. C. Levy

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives

1. To determine if there are separate decarboxylases for spermidine and spermine synthesis.
2. To determine if this system for polyamine biosynthesis is the same when isolated from widely divergent sources.

Methods Employed

1. S-adenosyl-L-methionine decarboxylase activity determined by $^{14}CO_2$ release from S-adenosyl-L-methionine when either putrescine or spermidine is used as substrate. When putrescine is used as the substrate, there is spermidine formation. When spermidine is used as the substrate, there is spermine formation.
2. Sephadex gel filtration and fractionation.
3. DEAE-cellulose column chromatography.
4. Calcium hydroxylapatite column chromatography.
5. Protein determinations according to the method fo Lowry et al.
6. Molecular weight determinations by the methods of Andrews and Martin and Ames.

Major Findings

S-adenosyl-L-methionine decarboxylase has been purified more than 350-fold from rat liver by a new procedure. It was found that 40% saturation with ammonium sulfate actually caused a loss of between 60-90% of enzyme activity. These results are in contrast to the effect of ammonium sulfate recently reported on the enzyme purified from ventral prostate. The molecular weight of the enzyme as determined by Sephadex gel filtration and by sucrose density centrifugation was found to be approximately 50,000. Enzyme activity was inhibited by sulfhydryl group inhibitors and by inhibitors of pyridoxal

phosphate systems. Spermidine could be substituted for putrescine at all stages of purification, but there was always a 2:1 preference for putrescine. A similar type of enzyme has been isolated from sea urchins (L. pictus) and from L_{1210} cells grown in culture. The molecular weight of these enzymes ranges between 50,000-59,000, which seems very similar to that found for the liver system. The enzyme from sea urchins is inhibited by those substances affecting the rat liver system. At present electrophoresis is being used to determine if the enzyme will separate into subunits.

Significance to Cancer Research

Since the polyamine biosynthetic pathway is intimately tied with rapid growth, an understanding of the enzymes of the polyamine biosynthetic pathway would enable us to better elucidate inhibitors of the rapid growth process. With a purified enzyme system, one can test inhibitors that might be of value in cancer chemotherapy.

Proposed Course

To examine in detail if the enzyme isolated from L_{1210} cells differs in any way from other biological material. And to determine if these differences can be used as a means of detecting cancerous systems.

Publications

Feldman, M. J., Levy, C. C., and Russell, D. H.: The purification of S-adenosyl-L-methionine decarboxylase from rat liver: Inability to separate decarboxylase from spermidine synthesis. Biochem. Biophys. Res. Comm. 44: 675, 1971

Feldman, M. J., Levy, C. C., and Russell, D. H.: Purification and characterization of S-adenosyl-L-methionine decarboxylase from rat liver. Biochemistry 11: 671-677, 1971

Serial No. NCI-6290
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of a Sensitive Method for Polyamine Determination Utilizing Gas Liquid Chromatography

Principal Investigator: D. H. Russell

Other Investigators: L. J. Marton
C. C. Levy

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives
To develop a rapid and sensitive method for putrescine, spermidine and spermine quantification utilizing gas liquid chromatography.

Methods Employed
1. Assays were performed with a Packard Model 7400 gas liquid chromatography with hydrogen flame ionization detectors. Various packing materials for 4-ft coiled glass columns with a 2 mm internal diameter were used. It was determined that 3% OV-17 on 100-120 mesh Gas Chrom Q (Applied Science Laboratories) was the ideal packing material for optimal separation of derivatized polyamines. For good separation the polyamines had to be derivatized by reaction with trifluoroacetic anhydride.

Major Findings
A rapid and sensitive method for polyamine determination was developed using gas liquid chromatography. Accurate determinations of nanogram quantities of putrescine, spermidine and spermine were made after an assay time of approximately 15 minutes. As an internal standard for the gas liquid chromatography, 1-7 diaminoheptane was found to be ideal. This compound has a retention time between putrescine and spermidine so that by using the internal standard at the beginning of the extraction, the recovery rate of each polyamine could be determined.

Significance to Cancer Research
This method can be used to assay polyamine levels in cerebral spinal fluid,

in urine, or in blood. Since the method is rapid and extremely sensitive, large scale screening for polyamines in various body fluids can be undertaken. Thus, the development of this method should serve to validate previous findings of polyamines as indicators of neoplasia.

Proposed Course

The method of assaying for polyamines in various body fluids will be continued. The rapidity of the assay as well as its sensitivity should allow for the examination of a large population of normal and cancer patients. An attempt will be made to relate the amount of polyamines found in the cancer patients with their tumor burden.

Publications

Marton, L. J., Denton, M. D., McVicker, T. A., Levy, C. C., and Russell, D.H.: A sensitive method for the determination of polyamines utilizing gas liquid chromatography. Anal. Biochem. in press

Serial No. NCI-6291
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Polyamine Metabolism in Spleens and Livers of Leukemic Mice. Effects of Potential Anticancer Agents

Principal Investigator: O. Heby

Other Investigators: D. H. Russell

Cooperating Units: None

Man Years: (July 1, 1971 through June 30, 1972)

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives

To define the functions of putrescine, spermidine, and spermine, and to understand possible control mechanisms of their synthesis in leukemic mice.

Methods Employed

1. Spectrophotometric quantitation of putrescine, spermidine, and spermine in tissues after homogenization in acid, alkaline butanol extraction, high voltage electrophoretic separation and ninhydrin staining.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine- and spermine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine (carboxyl-^{14}C).

Major Findings

A study conducted during the first month as a Visiting Fellow indicates that the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities are elevated in spleens of leukemic mice 24 hours after interperitoneal L_{1210} leukemia cell inoculation. The ornithine decarboxylase activity decreased rapidly and was normal on day 2 of tumor growth, whereas the S-adenosyl-L-methionine decarboxylase activity remained elevated during the first 8 days of tumor growth. The maximal survival of leukemic mice was 9 days. It was shown that 5-azacytidine markedly suppressed the enzyme activities in leukemic mice when injected daily (day 1-9). The drug injections were started one day after tumor transplantation, in order to certify 100% tumor

"takes." Except for the effects on the enzymes, the drug treatment caused a 300% increase in life span of the host mice. At the time when the drug treatment was interrupted, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities increased rapidly both in drug-treated leukemic mice and in drug-treated control mice. indicating that 5-azacytidine exerts a general suppression on polyamine-synthesizing enzymes.

Significance to Cancer Research

An understanding of the early events that occur upon an invasion of malignant cells is necessary in order to structure effective chemotherapy.

Proposed Course

1. Continued experiments to confirm the results obtained.
2. Since it has been shown (Hofer and Hofer, Cancer Res. 31, 402, 1971) that implantation of L_{1210} interperitoneally is followed by rapid migration of tumor cells from the injection site to other organs, it would be important to study the ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities in spleens and livers of mice every 2-3 hours following the inoculation of L_{1210} leukemia cells. L_{1210} metastasis is very high during early phases (first 24 hours) of tumor growth, and much slower in advanced tumors, and it might be possible to correlate early changes of enzyme activities in spleen and liver with the reported invasion of tumor cells in these organs.
3. Regular screening of polyamine synthesis and accumulation before, during, and after chemotherapy by various potent agents, to determine if polyamine levels at any given point in therapy can serve as a reliable indicator for future chemotherapy, i.e. best possible dosage schedule, when to stop, when to continue themotherapy.
4. It would be useful to obtain data on the cellular composition of the spleen during tumor growth and chemotherapy, to understand what causes the marked weight changes observed, and in order to, if possible, distinguish between enzymatic changes in metastatic leukemic cells and other kinds of cells.

Publications - None

Serial No.: NCI-6020

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanisms of Human Lymphocyte Reactivity to Non-Self

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Leonard Chess, M.D.
Carl Levy, Ph.D.
Morton Schmukler, M.D.
Kendall A. Smith, M.D.

Cooperating Units: Section of Enzymology and Drug Metabolism

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To enhance the sensitivity of immunologic induced blastogenesis in vitro.

Methods Employed:

Short-termed tissue cultures in which the proliferative response of cells in question were monitored by measuring the incorporation of tritiated thymidine.

Major Findings:

Two agents were found to have contrasting effects on sensitized lymphocyte induced proliferation in vitro to specific antigen. In addition, these agents were found to have a contrasting effect both in the response of non-sensitized cells to allogeneic lymphocytes as well as the response of lymphocytes to phytohemagglutinin. The first agent, adamantanamine hydrochloride was shown to enhance the reactivity of phytohemagglutinin at concentrations which simultaneously suppressed the reactivity of the same cell population when exposed to specific antigen. In contrast, amplification of the response of sensitized cells to specific antigen as well as the re-

Major Findings (continued):

sponse of normal cells to allogeneic lymphocytes was brought about by the presence of a synthetic polyribonucleotide complex, polyadenylic-polyuridylic acid. Alternatively, PHA stimulation was depressed by this polyribonucleotide. The latter compound was shown to enable sensitized cells to respond to an antigenic dose not stimulatory in its absence. The kinetics of cell proliferation was not altered by polyadenylic-polyuridylic acid, and non-sensitized lymphocytes could not be induced to respond to specific antigens in its presence. In contrast to the amplification of responses to antigens, polyadenylic-polyuridylic acid depressed the response to PHA over a wide range of concentrations.

Relevance:

1. Adamantanamine hydrochloride possesses unique immunosuppressive characteristics in that it inhibits the response of lymphocytes to specific antigen while not inhibiting other proliferative capabilities, i.e., the response of phytohemagglutinin. This characteristic may be useful in characterizing those responses in vitro which are immunologically specific as compared to those which are not.

2. The synthetic polyribonucleotide complex, polyadenylic-polyuridylic acid significantly enhances the sensitivity of leukocyte cultures. This complex may be a useful adjunct to culture systems designed to assay lymphocyte immune responsiveness to weak antigens.

Proposed Course:

To further evaluate these phenomena and apply them to current projects in which the measurement of host recognition of non-self is required.

Honors and Awards: None

Presentations:

Seventh Leukocyte Culture Conference

Publications:

Mardiney, M.R., Jr., and Bredt, A.B.: The Immunosuppressive Effect of Amantadine Upon the Response of Lymphocytes to Specific Antigens in Vitro. Transplantation 12:183, 1971.

Serial No.: NCI-6248
1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: To Determine the Effect of Freezing on the Transformation of Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigens and PHA.

Previous Serial Number: SAME

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Leonard Chess, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

Previous work has defined that human leukocytes frozen at different times and stored for various times transform in a reproducible manner when placed in culture with PHA or as one population of the two-way or the one-way mixed lymphocyte reaction. In addition, previous unreactivity of frozen-stored lymphocytes when cultured with specific antigen or as both partners in the mixed lymphocyte reaction were shown to be reconstituted by the addition of fresh autologous or homologous plasma. Continued experimentation with this system was designed to define those parameters whereby optimum response could be obtained and to define whether quantitative reproducibility to specific antigens or to frozen-stored leukocytes in the one-way mixed lymphocyte reaction could also be observed.

Methods Employed:

1. Slow freezing of lymphocytes in media containing 10% DMSO.
2. Short-term tissue cultures in which the proliferative response of the cells in question were evaluated by measuring the incorporation of thymidine.

Major Findings:

Lymphocytes from normal individuals were obtained at various periods of time and stored in a frozen state up to nine months in the vapor phase of a liquid nitrogen freezer. These cells were shown to respond reproducibly both quantitatively and qualitatively when cultured with specific antigens, allogeneic lymphocytes and PHA.

Proposed Course:

To utilize this technology to evaluate immune function in the normal and diseased host.

Honors and Awards: None

Presentations:

Sixth Leukocyte Culture Conference

Publications:

Chess, L., Bock, G.N., and Mardiney, M.R., Jr.: Reconstitution of the Reactivity of Frozen Stored Human Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigen. Proceedings of the Sixth Leukocyte Culture Conference, Academic Press, January, 1972.

Bock, G.N., Chess, L., and Mardiney, M.R., Jr.: The Prevention of Clumping of Frozen-Stored Leukocyte Populations by EDTA. Cryobiology, in press.

Serial No.: NCI-6249
1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients

Previous Serial Number: SAME

Principal Investigator: John C. Sutherland, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.
Roy Van Markham
Eugene Cimino
Walter P. Drake, M.S.

Cooperating Units: Department of Pathology, USPHS Hospital, Baltimore, Maryland

Man Years:

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

1. To determine the association of long-term viral disease and neoplasia in man.
2. To determine the probability of immune complex disease in mice considered immunologically tolerant to oncogenic virus.

Methods Employed:

1. We evaluated at autopsy the kidneys of 93 patients with lymphoma and leukemia for evidence of immune complex disease. 29 kidneys from patients who died without cancer or evidence of glomerulonephritis served as controls (C). Kidneys were stained with fluoresceinated antibodies to gamma and B_{1C} globulins, albumin and fibrinogen. Glomeruli of kidneys from 18 L & L and 7 C patients were positive for gamma globulin and of these, 8 and 2 respectively, for B_{1C} globulin. Antisera to the following viruses have been used to identify viral antigen in 6 L & L and 3 C patients: polyoma, adenovirus, reo-III, Epstein-Barr virus, herpes 1 and 2, cytomegalo-, fowl leukemia-, murine sarcoma-, and

Methods Employed (continued):

feline leukemia viruses (FeLV) and Mycoplasma pneumoniae.

2. Glomeruli of 8-10 month old AKR mice were examined by immunofluorescence for the presence of immune complex disease. Kidneys were stained with antisera to mouse gamma and B_{1C} globulins and albumin. In addition, the kidneys were stained with the following viral antisera: rabbit anti-fowl myeloblastosis virus containing group specific activity against avian leukemia gs antigens, guinea pig anti-cytomegalovirus, rabbit anti-ether disrupted feline leukemia virus absorbed in vivo in a kitten, guinea pig anti-SES disrupted FeLV and two rat serums from Fisher rats bearing transplanted tumors induced by Moloney sarcoma virus.

Major Findings:

1. Human Study:

Glomeruli of 2 patients with acute myelocytic leukemia were positive for FeLV in a distribution similar to that observed for gamma and B_{1C} globulins. Cat tissue and human liver powder absorption as well as absorption in vivo in the cat did not diminish positive fluorescence. These findings indicate that immune complex disease initiated by an oncornavirus-like antigen may be associated with leukemia in man.

2. AKR Model:

In all kidneys tested, the glomeruli stained positively for mouse IgG and B_{1C} globulins. Positive fluorescence existed both within capillary loops and mesangial areas and was granular in type. No positive fluorescence was seen when glomeruli were stained for mouse albumin. Glomeruli were also positive when stained by the indirect method with rabbit anti-ether disrupted FeLV, guinea pig anti-SDS disrupted FeLV, and both rat antisera against MuLV gs antigens. No staining was noted with the rabbit anti-fowl myeloblastosis virus, guinea pig anti-cytomegalovirus or normal rat serum. In addition, a blocking experiment was performed in which one antiserum possessing specificity to both MuLV gs-1 and gs-3 was used after an antiserum to only the gs-3 determinant indicated that MuLV gs-1 determinants were also present in these complexes.

Relevance:

These observations suggest that a careful study of patients with neoplasia may define host response to viral agents, of which at the moment, we are unfamiliar. This may also allow for the determination of host response to tumor antigens. Such a determination has been made by Lewis et al (Lancet 2:134, 1971) in which immune complex disease was found in a patient with bronchial carcinoma. In this case, the complexes consisted of specific antibody and tumor antigen. Such studies are of great importance since they

Relevance (continued):

relate intimately to the etiology as well as pathogenesis and treatment of neoplasia.

Proposed Course:

1. To continue to screen for antigen in these complexes
2. To continue to elute antibody from these kidneys and test for specificity
3. To continue screening patients for this type of injury.

Honors and Awards: None

Presentations:

First Meeting of the European Division of the International Society of Hematology

Institut de Cancerologie et D'Immunogenetique
Hopital Paul Brousse
Paris, France

American Association for Cancer Research, 1972

Publications:

Markham, R.V., Sutherland, J.C., Cimino, E.F., Drake, W.P., and Mardiney, M.R., Jr.: Immune Complexes Localized in the Renal Glomeruli of AKR Mice: The Presence of MuLV gs-1 and C-Type RNA Tumor Virus gs-3 Determinants. Europ. J. Clin. Biol. Res., in press.

Sutherland, J.C., and Mardiney, M.R., Jr.: The Presence of Leukemia Virus (Feline) Like Antigen in Antigen-Antibody Complexes Localized in Glomeruli of a Patient with Acute Myelogenous Leukemia. First Meeting of the European Division of the International Society of Hematology. December, 1971.

Sutherland, J.C., and Mardiney, M.R., Jr.: Analysis of Immune Complex Disease in Leukemia and Lymphoma Patients: The Presence of an Oncornavirus Related Antigen. American Association Cancer Research, 1972.

Serial No.: NCI-6273

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Bacterial Contamination in Platelet Products Stored at 25° C

Previous Serial Number: SAME

Principal Investigator: Donald H. Buchholz, M.D.

Other Investigators: Viola Mae Young, Ph.D.
Michael R. Mardiney, Jr., M.D.

Cooperating Units: Central Blood Supply; Baltimore Red Cross

Man Years:

Total:	7/10
Professional:	1/5
Other:	1/2

Project Description:

Objectives:

Recent reports have suggested improved post-transfusion platelet survival of platelet products prepared and stored at 25° C rather than 4° C. An objection to 25° C storage is the possibility of proliferation of bacteria introduced inadvertently into blood collection systems during phlebotomy or component preparation. Following two instances of gram-negative septicemia in cancer patients transfused with such stored platelets, a study was begun in 1971 to determine the frequency of bacterial contamination in platelets stored at 25° C.

Methods Employed:

Platelet concentrates were obtained from commercial and volunteer sources and were pooled in groups of 8 to 10 units after storage at ambient temperature for up to 96 hours. Each pool was composed of platelets obtained from a common source and stored a similar length of time prior to culture. A sample of each pool was inoculated into tryptic soy broth, brain heart infusion and thioglycollate broth which were incubated at 37° C. Later in the study, duplicate cultures were also done at 25° C. Precautions were taken to prevent contamination during pooling or culturing. The degree of

Methods Employed (continued):

contamination in platelet pools was roughly quantitated by applying one-half ml specimens to blood agar plates and counting the number of colonies present after 96 hours. Small samples obtained from individual bags of fresh platelets were also cultured upon arrival in an attempt to detect contaminated units before use. Samples from unused bags kept at room temperature for from 11 to 71 days were gram-stained and cultured to determine the frequency of bacterial presence in units not subject to manipulation (pooling) and the possibility of exogenous entry of microorganisms. Additionally, the first 10 ml of blood from 157 phlebotomies was diverted into culture bottles to evaluate the role of skin "coring" by needles as a possible mode of bacterial entry. Cultures were examined frequently for evidence of bacterial growth; all apparently negative cultures were routinely entered and subcultured on two occasions. Bacteria were identified according to standard methods.

Major Findings:

Culture of 4560 units of platelets in 566 pools revealed bacteria in 74 pools. While Staphylococcus epidermidis and diphtheroids were most frequently isolated, other bacteria, including pathogenic organisms such as Staphylococcus aureus and Enterobacter cloacae were also found. A total of four patient septicemias have been directly due to infusion of ambient temperature stored platelets containing E. cloacae (2), Flavobacterium sp. and Serratia sp. The recovery of bacteria from platelet pools increased as time of storage increased, with 0.9% of fresh, 2.1% of one day old, 2.5% of two day old, and 4.3% of three day old platelet units calculated to contain bacteria. Most pools contained only a few bacteria per ml, as demonstrated by blood agar plate cultures of a known volume of the pool, although several pools were found to harbor greater than 1000 microorganisms per ml. Culture of fresh specimens obtained from the tubing of individual bags of platelet concentrate revealed bacteria in 1.4% of 3251 samples tested. Thirty-eight of 631 (6.0%) individual bags of platelets kept at ambient temperature for up to 71 days contained bacteria; when large numbers of bacteria were present, the microorganisms could also be seen on gram-stained specimens obtained directly from the bag. Diversion of the initial 10 ml of blood from donors after venipuncture showed five of 157 samples to contain bacteria (four S. epidermidis, one Bacillus sp.), suggesting a possible mode of bacterial entry in some instances.

Proposed Course:

At the present time, attempts are being made to transfuse fresh platelets whenever possible.

Honors and Awards: None

Publications:

Buchholz, D.H., Young, V.M., Friedman, N.R., Reilly, J.A., and Mardiney, M.R., Jr.: Bacterial Proliferation in Platelet Products Stored at Room Temperature. New Eng. J. Med. 285:429-433, 1971.

Buchholz, D.H., Young, V.M., Friedman, N.R., Reilly, J.A., and Mardiney, M.R., Jr: Detection and Quantitation of Bacteria in Platelet Products Stored at Ambient Temperature. Submitted for publication.

Serial No.: NCI-6274

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody

Previous Serial Number: 6274 and 6019

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Peter C. Ungaro, M.D.
Walter Drake, M.S.

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	1
Other:	1 1/2

Project Description:

Objectives:

One of the main obstacles in tumor immunology is the inability to differentiate the presence or absence of antigens upon neoplastic cells which are qualitatively or quantitatively specific for that cell. This is especially true if immunization takes place in a xenogeneic host. Previous work has allowed us to develop a method for the production of antibody to tumor cells which has bypassed some of the problems described. The technique is based upon the principal that passively administered antibody can specifically inhibit production of similar antibody within hosts of the same species or outside that species. Briefly, the model studied previously consisted of a murine C57BL/6 leukemia, E.L.4, to which specific antibody was produced in the rabbit only by simultaneous injection of a rabbit antibody to normal C57BL/6 spleen cells. The present work tested the effect of such immunization in burros. The exploration of large animal use was designed specifically for further possible tests of the production of tumor specific antibody to human cells.

Methods Employed:

Anti-C57BL/6 spleen cell sera was produced in normal burros by the IV in-

Methods Employed (continued):

jection of 1 x 10^9 nucleated spleen cells weekly for a period of seven weeks. At the eighth week, the antisera was harvested. This antisera was then incubated in excess with 1 billion E.L.4 cells and both cells and antisera were utilized to immunize normal burros on days 0, 14, 21 and 28. Sera was harvested on day 35. The antibody derived from these animals was then evaluated for specificity to E.L.4.

Major Findings:

The present work demonstrated that while specific antibody against E.L.4 leukemia could be produced using the passive administration of antibody to normal splenic antigens and the administration of E.L.4 leukemia, no such marked differentiation could be observed in the burro. The burros response was unique in that E.L.4 cells alone elicited a anti-E.L.4 response. Although this response was relatively specific for the E.L.4 absolute specificity could not be fully defined. The use of passively administered antibody, however, to normal cellular antigens in this situation, markedly diminished cross reactivity to spleen cells in relation to the activity present directed primarily to E.L.4 cells.

Relevance:

The above work again defines that antibody production to cellular antigens can be altered by the simultaneous administration of homologous antibody to determinants common to tumor and normal cells. This approach will allow the defining of antigens both to normal and neoplastic cells which cannot be ascertained under normal immunization conditions. It is not predicted that antibody response to new antigens will occur in all situations in which this approach is utilized, but it is anticipated that a significant yield may accrue from such an approach.

Proposed Course:

1. To further utilize this approach to define tumor specific antigens on neoplastic cells.
2. To evaluate therapeutic use of such antisera.
3. To extend this approach in defining tumor specific antigens on human neoplastic cells.

Honors and Awards: None

Presentations: None

Publications:

Ungaro, P.C., Drake, W.P., Buchholz, D.H., and Mardiney, M.R., Jr.: Alterati of Specificity of Anti-Tumor Antisera by the Use of Passively Administered

Publications (continued):

Antibody. Cancer Res., in press.

Drake, W.P., Ungaro, P.C., and Mardiney, M.R., Jr.: Preservation of Cellular Antigenicity of Tumor Cells by the Use of Formalin-Fixation. Cancer Res., 32:1042-1044, 1972.

Drake, W.P., Ungaro, P.C., and Mardiney, M.R., Jr.: Formalin-Fixed Cell Preparations for Use in the Automated Trypan Blue Cytotoxic Assay. Submitted for publication.

Serial No.: NCI-6275

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cell Transfusion Therapy

Previous Serial Number: SAME

Principal Investigator: Donald H. Buchholz, M.D.

Other Investigators: Peter H. Wiernik, M.D.
Michael R. Mardiney, Jr., M.D.

Cooperating Units: Baltimore Red Cross; Central Blood Service

Man Years:

Total:	2
Professional:	3/4
Other:	1 1/4

Project Description:

Objectives:

With the advent of newer aggressive chemotherapeutic approaches to the treatment of malignancy, problems arising from secondary suppression of the bone marrow have become more prominent and have led to increased risk of infection or hemorrhage. Replacement therapy should substantially decrease these patient risks.

Methods Employed:

I. Platelet Transfusion

Platelets are purchased from the Baltimore Red Cross and from the Central Blood Service. Platelets are prepared and stored at ambient temperature for up to 48 hours. Pre-transfusion and 1-, 3-, and 12 hour post-infusion recipient specimens are obtained to evaluate patient platelet response. Additionally, 400 units of platelets were frozen in 5% Dimethylsulfoxide and stored at -196° C until needed for emergency transfusion.

II. White Blood Cell Transfusion

Granulocytes may be obtained from donors through the use of the NCI-IBM Blood

Methods Employed (continued):

Cell Separator. This machine semi-selectively separates granulocytes from donor blood by means of differential centrifugation by repeatedly circulating donor blood through the machine. A more efficient means of granulocyte collection is the use of a nylon fiber filter (Leuko-Pak) which selectively traps granulocytes in fresh heparinized blood. By adapting the filters to a continuous flow system, large volumes of donor blood may be passed through the filters in relatively short periods of time.

Major Findings:

I. Platelet Transfusion

Platelets prepared and stored at ambient temperature were used for pa ient transfusion. However, due to the risk of bacterial proliferation in units stored at this temperature, most transfusions were given fresh. No major findings have been made as yet but the program is still ongoing.

II. White Blood Cell Transfusion

Granulocyte transfusions have been given both for prophylaxis against infection and for treatment of documented septicemia. Most donors have been HL-A typed and selected to be those most compatible with the recipient. Although data is not available for all granulocyte donations, the use of the Leuko-Pak rather than the NCI-IBM Blood Cell Separator allows significantly greater numbers of granulocytes to be collected. Using the Cell Separator, and average of 5.7×10^9 granulocytes (range 1.4 to 10.9×10^9) were collected during 18 donations in an average donation time of 196 minutes (range 150 to 245 minutes) for an average yield of 1.74×10^9 granulocytes per hour of donation. Cells collected in this manner contain about an equal number of contaminating lymphocytes and monocytes. Cell yield with the Leuko-Pak was proportional to the volume of blood passed through the filters and to the number of filters used during a donation. Total granulocytes collected in this manner (33 donations) depended upon filter arrangement and ranged from 10.1×10^9 to 59.8×10^9 granulocytes during an average donation time of 137 minutes (range 85 to 195 minutes). The yield of granulocytes per hour of donation time ranged from 7.1×10^9 granulocytes per hour using two filters to 19.9×10^9 granulocytes per hour when six filters were used. A significant number of granulocytes passed through the filters during a donation because of preferential blood flow paths and consequent fiber "saturation". If the filters are washed to return red blood cells to the donor, granulocytes are removed from the filter in proportion to the volume of wash solution (normal saline containing 5% bicarbonate solution and heparin, pH approximately 7.7). After elution of collected granulocytes by passing an ACD-plasma-ACD-saline mixture, pH approximately 6.5, through the filters, an average of 93% of cells harvested (range 86% to 99%) are granulocytes. Such cells are 97 to 100% viable by trypan blue dye exclusion test and are capable of *in vitro* phagocytosis and bacteriocidal

Major Findings (continued):

activity.

Proposed Course:

I. Platelet Transfusion Program

Platelet transfusions will continue to be given patients who become thrombocytopenic as needed consequent to chemotherapy. If and when adequate laboratory personnel are provided to evaluate such transfusions, such evaluation will be initiated. The plateletpheresis program utilizing HL-A typed family members as platelet donors will be continued as needed for those patients who become refractory to random donor transfusion. Such a program will be expanded as adequate personnel become available.

II. White Blood Cell Transfusion

In as much as the use of the Leuko-Pak for granulocyte collection allows from 5 to 15-fold more granulocytes to be collected per man hour of physician and nurse operator time, this system will continue to be evaluated as a means of easy procurement of granulocytes. The use of such cells will be evaluated by clinical observation of patients and responses to septicemia or localized infection by *in vitro* techniques to evaluate phagocytic and bacteriocidal capacity of such cells, and by electron microscopic evaluation of cellular morphology following exposure to the nylon fiber filter.

Honors and Awards: None

Publications: None

Serial No.: NCI- 6292

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunologic Effects of BCG in Man

Previous Serial Number: None

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Leonard Chess, M.D.
Gerald N. Bock, M.D.
Peter C. Ungaro, M.D.
Donald H. Buchholz, M.D.

Cooperating Units: None

Man Years:

Total: 1 1/2
Professional: 1
Other: 1/2

Project Description:

Objectives:

The purported effect of BCG (Bacillus-Calmette-Guerin) in the non-specific activation of immunologic and reticuloendothelial function is the basis for its present use in the active immunotherapy of malignant disease in man. The present study describes a detailed analysis of immune function in four patients with previously untreated metastatic melanoma prior to and during the administration of BCG by weekly escarifications.

Methods Employed:

Immune function was assessed by (1) the in vitro transformation of frozen-stored lymphocytes in response to PHA, PPD, SKSD, tetanus toxoid (TT) and allogeneic lymphocytes measured by ^{3}H thymidine incorporation (2) quantitative C'3, IgG, IgA, IgM levels determined by immunodiffusion and (3) skin testing. The data was correlated with the clinical course.

Major Findings:

There was no change in the IgA, IgM or C'3 levels. In contrast, significant increases in IgG levels were found in three of four patients. The in vitro

Major Findings (continued):

lymphocyte response to PPD, SKSD, and TT was significantly augmented during the administration of BCG despite ltttle change in response to PHA or allogeneic lymphocytes. It is concluded that (1) BCG increases the immune response of sensitized cells to specific antigens, i.e. PPD, SKSD, and tetanus toxoid; (2) BCG has little or no effect on a primary immune response in vitro, i.e. the response to allogeneic leukocytes (3) BCG has little or no effect on the response of cells to PHA and; (4) BCG selectively increases IgG levels without enhancing other IgA, IgM or C'3.

Relevance:

If BCG or like agents are to play a substantial role in the therapy of neoplasia, their effectiveness may be expected to be greatest in those situations in which host immunologic response to tumor is present. If that response is not present at all, these agents may be of little or no benefit.

Proposed Course:

To further evaluate agents capable of enhancing host immunologic response.

Presentations:

American Society of Hematology

Institut de Cancerologie et D'Immunogenetique
Hopital Paul Brousse
Paris, France

Colloquium of the C.N.R.S. on "The Investigation and Stimulation of Immunity in Cancer Patients"
Institut de Cancerologie et D'Immunogenetique
Paris, France

Publications:

Chess, L., Bock, G.N., Ungaro, P.C., Buchholz, D.H., and Mardiney, M.R., Jr.: Immunologic Effects of BCG in Patients with Malignant Melanoma. Abstract, Annual Meeting American Society of Hematology, December, 1971.

Serial No.: NCI-6250

1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Ultrastructural and Tissue Culture Studies of Acute Leukemic Cells of Patients Before and During Treatment

Previous Serial Number: SAME

Principal Investigator: Frances T. Sanel

Other Investigators: Peter H. Wiernik, M.D.

Cooperating Units: Baltimore Cancer Research Center, Tumor Service

Man Years:

Total:	1/4
Professional:	1/4
Other:	1/2

Project Description:

Objectives:

Continuing studies are directed toward determining the responsiveness to and cytological aspects of phagocytosis provoking agents such as thorium dioxide, and viable bacteria on peripheral blood and bone marrow cells from patients with acute leukemia before and during treatment. Concommitant search for virus is pursued.

Major Findings:

Aberrant cytomorphology and deficiency of lysosomal enzymes in leukemic cells have been observed. Myeloperoxidase deficient leukemic granulocytes from a patient with myelomonocytic leukemia were replaced by a population of cells with normal amounts of this enzyme after chemotherapy. Although the ability to phagocytose test material was not impaired in the untreated state, lysosomal breakdown of ingested material was not observed.

Proposed Course:

1. Continued study of monocytic and myelomonocytic leukemias with phagocytosis provoking agents.

Proposed Course (continued):

2. Cytochemical study of the glycocalyx of leukemic cells and utilization of freeze-etch techniques to elucidate the "zipperlike junctions" formed by some leukemic cells under test conditions.
3. Soft agar tissue culture to induce differentiation of leukemic stem cells.

Publications:

Sanel, F.T. and Wiernik, P.H.: Myeloperoxidase Deficient Leukemic Granulocytes: An Electron Microscopic Study (in preparation).

Serial No.: NCI-6272

1. Baltimore Cancer Research Center
2. Clinical Branch
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Myelomonocytic Leukemia in an Animal Model System: Electron Microscopy and Biological Experiments

Previous Serial Number: SAME

Principal Investigator: Frances T. Sanel

Other Investigators: None

Cooperating Units: Laboratory of Viral Diseases (Dr. Janet Hartley)

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To study etiology, passaging under varying conditions and trans-species grafting of transplantable, muramidase producing myelomonocytic WEHI-3 tumor cells utilizing techniques of short term tissue culture, hematological, histological, histochemical and ultrastructural techniques.

Major Findings:

Muramidase-producing tumor cells contained peroxidase positive granules additionally confirming their identity as non-lymphoid precursors.

We identified "C" type viral particles by electron microscopy in tumor cells in spleen, liver, and bone marrow. They were tested as tumor extracts by complement fixation and XC mouse embryo cytopathogenicity tests and they contained the group specific antigen (gs) of murine leukemia viruses and demonstrated high levels of infectivity. Recent results of cell-free passage into newborn Balb/c mice reveal massive production of "C" type viruses after a four month latency period. The disease in these animals differs from that in donor mice and remains to be characterized.

Major Findings (continued):

Trans-species passage of Balb/c tumor cells into newborn inbred Fisher rats resulted in induction of a transplantable lymphoid leukemia with massive thymomas, mesenteric tumors, enlarged spleens and systemic dissemination of leukemic cells. "C" type viral particles in tumor extracts examined by complement fixation and XC tests contained the gs antigen of MuLV and also demonstrated very high titers of infectivity.

Transfer of tumor cells from Fisher rats into newborn Balb/c mice induced lymphoid neoplasia in the latter.

Significance to Cancer Research

These animal systems could be valuable models for the study of etiology, diagnosis and therapy of human leukemia.

Proposed Course:

1. Continued study of the oncogenic potential of the "C" type particles.
2. Characterization of this disease in Balb/c mice induced by cell-free filtrates.
3. Histochemical localization of muramidase within the myelomonocytic cells.
4. Therapeutic studies on the WEHI-3 Balb/c animals in collaboration with Dr. M. Walker.
5. Freeze etch studies of virus producing cells.

Publications:

Sanel, F.T.: Virus-Producing Cells in WEHI-3 Myelomonocytic Tumors. Proc. Amer. Assoc. for Cancer Res., 12:76, 1971. Abstract No. 301.

Sanel, F.T.: Development of Lymphocytic Leukemia in Rats Implanted with Virus-Producing Myelomonocytic Cells from Balb/c Mice. Proc. Amer. Soc. Cell Biol., 258, Abstract No. 508

Sanel, F.T.: Infectious Murine Leukemia Viruses Produced by Myelomonocytic Tumor Cells in Balb/c Mice: Ultrastructural and Biological Studies (in preparation).

Sanel, F.T.: Transplantable Lymphoid Leukemia Induced in Rats by Myelomonocytic Mouse Cells Producing Infectious "C" Type Particles (in preparation)

ANNUAL REPORT

CANCER THERAPY EVALUATION BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

Office of the Chief

The Cancer Therapy Evaluation Branch is responsible for coordinating and monitoring the clinical trials on the anticancer drugs that have demonstrated high activity in animals in the preclinical phase of the Chemotherapy Program. The Branch reviews and evaluates the cancer therapy activities of the various types of investigation involved in the clinical testing area. These include the expanding Clinical Contract Program, collaboration with the Veterans Administration, disease oriented task forces, cooperative groups and individual physicians. A continuing liaison is maintained with various advisory and consultant groups established as working committees to study the effectiveness of specific types and methods of cancer therapy. The clinical aspect of international liaison, which is increasing significantly with the opening of liaison offices in Japan and Brussels, is another responsibility of the Branch.

During the past year the Branch has expanded considerably with the formation of two new sections. The Drug Liaison and Distribution Section was formed with John Penta, Ph.D., as its Head. This section is responsible for the communication and filing of all information required by the Food and Drug Administration and the Division of Biological Standards in connection with the Drug Development Program of the National Cancer Institute. In addition, this section is responsible for the national worldwide shipment of investigational drugs, as well as the distribution of commercially available drugs, to the Cooperative Group Program.

The Clinical Trial Surveillance Section was formed with Mr. William Soper as its Head. This section is responsible for handling the massive amount of clinical data which comes into the Branch from various sources and in various formats. This section is attempting to devise a methodical approach to the organization of this data in such a way that it is both easily retrievable and available for the rapid integration and evaluation of new data. In this way, it is hoped that promising leads can be uncovered as quickly as possible.

During the past year, the Branch has become actively involved in the numerous total disease oriented approach programs evolving within the National Cancer Institute. It has attempted to integrate the drug oriented needs of the drug development part of Program into the basic philosophic approach of the entire Program

that cancer therapy must be multidisciplined and geared to the specific exigencies of individual tumors. In line with this, the Chief of the Cancer Therapy Evaluation Branch is a member of the following disease oriented groups:

1. The Chemotherapy Subcommittee, Lung Cancer Working Party
2. The Therapy Committee, Breast Cancer Task Force
3. Chemotherapy Committee, Colo-rectal Cancer National Program
4. Protocol Committee, Brain Tumor Study Group
5. Osteosarcoma Study Group
6. Chairman, Testicular Tumor Working Group

Traditionally, the clinical evaluation of a new antitumor drug has been divided into three phases. This division into phases, while occassionally arbitrary, is a useful one for conceptually evaluating both clinical results and the methodology of clinical trials. The clinical evaluation of a new compound begins with trials which are almost completely drug oriented (phase I) and ends with trials which should be, in the main, disease oriented (phase III). It is in the phase II trials that a bridge between the drug oriented and disease oriented approach is both fused and crossed. The phase II trial is the most critical of all clinical trials because it is these trials which determine the future of a drug in any given tumor. In essence, phase II is the second screen in the Chemotherap Program's long drug development process. It is a screen because, ideally, one would like to test a new drug against a significant number of evaluable patients with every type of clinical cancer nd amenable to complete cure with other therapeutic modalities. Sinc this is not feasible due to the limited patient resourses availabl it is therefore necessary to pick "signal" tumors which will, hope fully, test the therapeutic potential of a new drug against both the so-called "fast growing" or "responsive" tumors, as well as th "slow growing" or less "responsive" tumors. Initially, the Progra had designated six such tumors. These were acute lymphocytic leukemia, acute myelocytic leukemia and undifferentiated lymphosarcoma for the fast-growing screen, and bronchogenic carcinoma, adenocarcinoma of the breast and adenocarcinoma of the large bowel for the slow growing screen. With the ability to expand clinical testing resources, it has been felt that new tumors should be added in the so-called slow growing category, as it is this group of tumors which constitutes a major area of increased program interes Among the tumors to be added to the list of those which will recei the highest priority for phase II clinical testing are adenocarcinoma of the pancreas and adenocarcinoma of the ovary. In addition major emphasis will be placed on rare tumors such as testicular ca cinoma and osteosarcoma, in which it is felt that drugs have a ver high potential for enhancing curative therapeutic attempts in the near future.

In recognition of the crucial importance of the phase II trial, a

great deal of effort has been put into the problems and methodology of this evaluation. In the past, these phase II trials were almost always an uncontrolled evaluation of 15-25 patients on a given disease with a new drug, or a massive study in 50-200 patients with all types of tumors, where obtaining an adequate number of patients on any given tumor was a matter of chance. Some of the problems inherent in the traditional phase II trial are seen when the data for a classic agent (5-fluorouracil) are looked at in one major tumor type (adenocarcinoma of the breast):

5-FLUOROURACIL IN BREAST CANCER

Standard Loading Dose
(15 mg/kg/d x 5, then 7.5 mg/kg E.O.D.)

Investigator	# Eval.	# Resp.	% Resp.
Ansfield et al.	676	156	23
Hall	22	15	65
Moore	56	18	32
Eastern Cooperative Oncology Group	30	8	26
Talley	30	11	36
Field	25	14	56
Eyerly	10	2	20
Vaitkevicius	28	7	25
Kennedy	43	18	44
Ravdin and Eisman	63	19	30
TOTAL:	983	268	27

Schedules Other Than
Standard Loading Course or Weekly

Investigator	Dosage Schedule	# Eval.	# Resp.	% Resp.
Nemoto and Dao	7.5 mg/kg/d x 10 or less if toxicity is seen	133	17	13

Hall	15 mg/kg/d by 1,2,4 or 8 hr. infusion	27	8	30
Mackman et al.	2.5 mg/kg q 6 H → 15 mg/kg/d x 5, then 1.25 mg/kg q 6 H → 7.5 mg/kg/d to toxicity	44	13	30
	TOTAL:	204	38	18

Weekly Administration

Investigator	Dosage Schedule	# Eval.	# Resp.	% Resp.
Jacobs et al.	15 mg/kg/wk x 4 then, if tolerated, 20 mg/kg/wk x 4	20	9	45
Horton et al.	7.5 mg/kg/wk	18	1	5
	15 mg/kg/wk	24	3	12
	20 mg/kg/wk	14	5	36
	TOTAL:	76	18	24
	Total (15-20 mg/kg/wk):	58	17	30

As can be seen, the response rates range from a low of 5% on one of the weekly schedules of Horton to a high of 65% in the study of Hall. If one removes the critically important aspect of dosage schedule and looks only at the data for the standard loading dose approach, there still exists a nearly three-fold difference betwee the lowest and highest reported response rates. Since in any scree the false negatives are the most disturbing, it becomes of great importance to attempt to elucidate these aspects of clinical trial design which lead to the wide range of therapeutic results as indicated above.

In approaching such a problem, a clinical trial can be divided into three variables: the state of the patient prior to therapy, the therapy itself and the state of the patient after therapy. It is the first variable, the state of the patient prior to therapy, which is most often neglected in phase II trials, as almost all of the emphasis is placed on the last variable, the state of the patient after therapy, in the form of a numerator/denominator response rate. A fascinating piece of data in support of the importance of patient selection comes from Dr. Marvin Schneiderman (Cancer Chemotherapy, Brodsky and Kahn, editors, p. 67-76, Year Book Medical Publishers, 1967), who has outlined the response to

chemotherapy of patients with lymphoma as correlated with their survival as estimated by the investigators prior to the onset of therapy. The study was a comparison of three alkylating agents and, for each agent, the response rate was dramatically higher for those with an estimated survival of greater than 9 months as compared for those of less than 6 months. The implications of this for phase II trials in a given tumor with only 20-30 patients are immediately obvious and not a little bit unsettling.

In breast cancer, a few papers have looked at some pretherapeutic variables in relation to response to 5-fluorouracil, and these may, in part, explain the variability of the published results. The Cancer Therapy Evaluation Branch has made a composite summary of the available data, which is shown below:

Cumulative Data on Breast Cancer Response to 5-Flourouracil Broken Down by Various Pretherapeutic Variables

Pretherapeutic Variable	# Pts. Eval.	# Obj. Regressions	% Resp
I. Menopausal Status			
1. 0-1 yr. post	97	32	33
2. 1-5 yr. post	141	25	17
3. 5-10 yr. post	80	15	19
4. > 10 yr. post	130	22	17
II. Disease Free Interval After Surgery			
1. 0-2 years	77	21	27
2. > 2 years	85	8	11
III. Site of Dominant Lesion			
1. Soft Tissue	167	49	30
2. Osseous	300	84	28
3. Visceral	650	117	18

As can be seen, a woman with a soft tissue dominant lesion, less than one year post menopausal with a disease free interval of less than two years, appears to have a much better chance of responding to 5-fluorouracil than does a woman with a visceral dominant lesion, more than one year post menopausal with a disease free interval greater than two years. When one adds to this brew the subjective ingredient of performance status or estimated survival, then it becomes obvious that a phase II trial of a new agent in breast cancer is not a simple drink which can be downed quickly if one is to have a clear head in the morning.

The Chemotherapy Program has attempted to alleviate some of the problems in phase II study by putting more structure into its

phase II study designs. Below is an outline of some of the designs which can be utilized:

I.	Drug A	Progressive Disease →	Drug B
II.	Drug A	Progressive Disease →	Drug B
	vs		
	Drug B	Progressive Disease →	Drug A
III.	Drug A	Progressive Disease →	Drug C
	vs		
	Drug B	Progressive Disease →	Drug C
IV.	Drug A	Progressive Disease →	Drug C
	vs		
	Drug B	Progressive Disease →	Drug D

It is felt that an optimal design might be a randomly allocated, controlled design with crossover at the time of progressive disease. An example of how this is being applied to breast cancer is shown in the design of a study currently under way at Memorial Hospital, sponsored by the Chemotherapy Program:

Stratification

1. Menopausal status
2. Disease free interval
3. Site of predominant lesion

RANDOM ALLOCATION

CCNU 130 mg/m^2 Q 6 wks. → PROGRESSIVE DISEASE → Cyclophosphamide 30 mg/m^2 Q 3 wks. or Daily

Adriamycin 25 $mg/m^2/d$ x 3 Q 3 wks. → PROGRESSIVE DISEASE → Cyclophosphamide

In the past, an institution with the ability to study two drugs per year in phase II study would have done it sequentially, putting 20 or so patients on Drug A and then 20 or so patients on Drug B. The Memorial Hospital design utilizes the same 40 or so patients to study the two drugs, but utilization of the controlled crossover design leads to a variety of advantages, outlined below:

1. Comparability of treatment groups.
2. Removal of patient selection bias.
3. Data on cross-resistance can be gathered.
4. Time to progressive disease can be utilized as an additional therapeutic endpoint.
5. Maximal utilization of patient resources.
6. Increased validity of survival as a therapeutic endpoint.

We now know that we will have comparability of treatment groups and some removal of patient selection bias. The utilization of pretherapeutic stratification can insure even greater comparability of groups through the even distribution of factors known to effect response.

The crossover aspect of the design enables us to gain crucial information about clinical cross-resistance between the agents in the study at no additional cost of patients. The Memorial Hospital design will give valuable information about cross-resistance between the nitrosourea drug, CCNU (which may act as an alkylating agent), and the standard alkylating agent, cyclophosphamide. In addition, maximal utilization of patient resources can be enhanced by comparing two schedules of cyclophosphamide in a controlled study which has not been done previously to our knowledge.

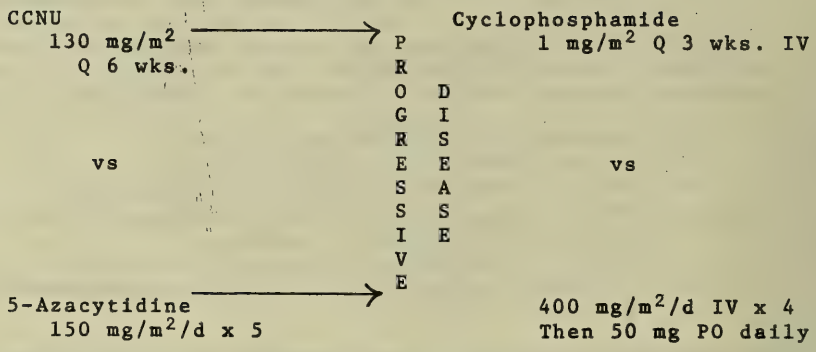
When the secondary therapy is fixed, one has still another advantage in that survival can be used as a therapeutic endpoint with increased validity, as it should reflect the initial therapeutic difference only. With this kind of study you can measure the percent achieving standard objective response and duration, and the percent achieving a no change or subjective benefit only status. In addition, one can look at a new parameter: the duration from onset of therapy to progressive disease, which would appear to me to be a parameter which should be directly related to drug effect, assuming the patients chosen are relatively homogeneous from a pretherapeutic evaluation point of view.

Below is the breast cancer protocol being sponsored at Roswell Park Memorial Institute and Albany Medical College:

<u>Stratification</u>

1. Menopausal status
2. Disease free interval
3. Site of predominant lesion

RANDOM ALLOCATION



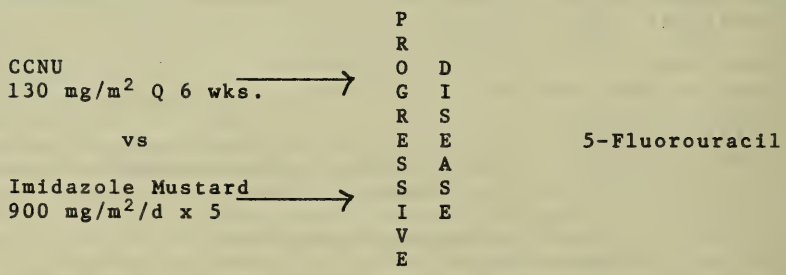
At the Mayo Clinic, two designs for breast cancer study have been tried, as shown below:

Stratification

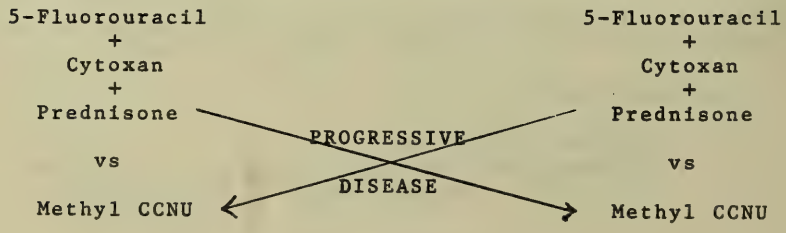
1. Menopausal status
2. Disease free interval
3. Site of predominant lesion

RANDOM ALLOCATION

Study I



Study II



In Study I, CCNU is run concomitantly with imidazole mustard (NSC 82196) and the crossover at the time of progressive disease is to 5-fluorouracil. Study II compares our newest nitrosourea, Methyl CCNU, to a three-drug combination of 5-fluorouracil, cyclophosphamide and prednisone. At the time of progressive disease, the two regimens cross over to each. This study will give information on the relative value of a combination regimen used as primary vs. secondary therapy.

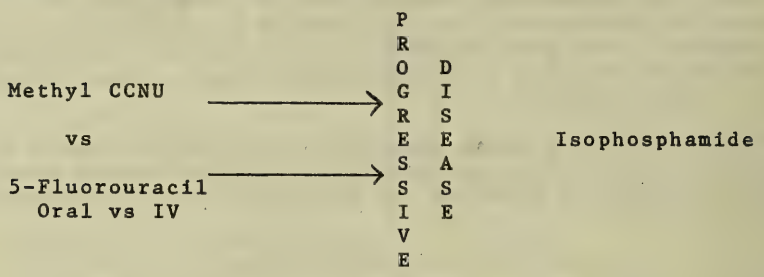
We are utilizing these designs in other solid tumors as well as in breast cancer. The pretherapeutic stratification used by our contract supported group at the Mayo Clinic for large bowel cancer is shown below:

1. Site of primary tumor
   a. Rectum
   b. Colon
2. Status of primary tumor
   a. Unaltered
   b. Resected
   c. Bypassed
3. Grade of anaplasia
   a. Low grade (Broder's 1,2)
   b. High grade (Broder's 3,4)
4. Indicator lesion
   a. Cutaneous or subcutaneous
   b. Abdominal
   c. Pulmonary
   d. Hepatic
   e. Other
5. Age
   a. < 50 years
   b. 50 - 65 years
   c. > 65 years
6. Disease free interval
   a. Simultaneous (< 1 month)
   b. 1 - 6 months
   c. 6 - 12 months
   d. > 1 year

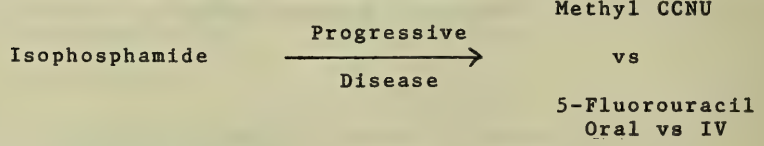
The experimental design utilized for the large bowel cancer evaluation is a two stage design. This study involves two stages and evaluates two new agents (methyl CCNU and isophosphamide) in comparison to 5-fluorouracil. All three drugs are used both as primary and secondary therapy, so that cross-resistance is randomized to oral vs. intravenous administration, so that further data of value should come out of this study. The study design is outlined on the following page:

RANDOM ALLOCATION

Stage I (30 untreated patients)



Stage II (15 untreated patients)



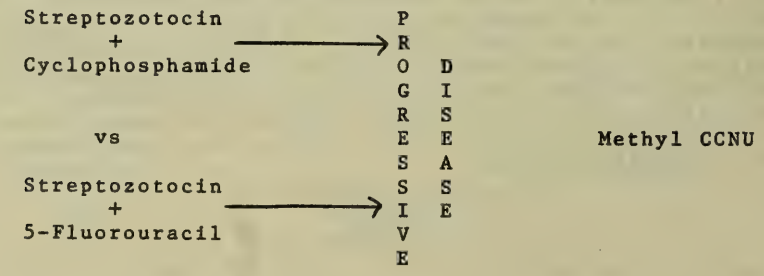
In pancreatic cancer, the Eastern Cooperative Oncology Group has also adopted a two-stage design, as outlined below:

Stratification

1. Site of Primary
   A. Head
   B. Body or Tail
2. Grade of Anaplasia
   A. Low Grade (Broder's Grade 1,2)
   B. High Grade (Broder's Grade 3,4)
3. Performance Status
   A. 50 or Less
   B. 60 or Higher

RANDOM ALLOCATION

Stage I (40 untreated patients)



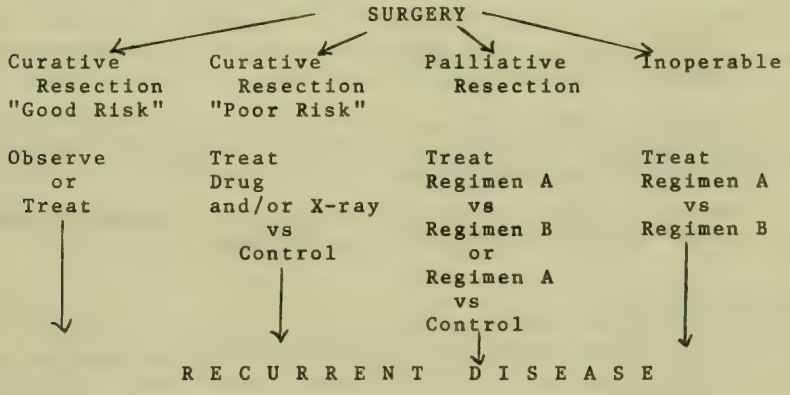
Stage II (20 untreated patients)

Methyl CCNU → Progression → Randomize

- A. Streptozotocin + Cyclophosphamide
- B. Streptozotocin + 5-Fluorouracil

One conclusion that emerges strongly from all of this discussion is the importance of phase II study designs and protocols geared for individual diseases. The problems of phase II study in acute leukemia are not the same as those encountered in studying bronchogenic carcinoma. For each disease, there needs to be a full elucidation of the meaningful pretherapeutic variables and a definition of areas that require stratification. The therapeutic results have to be evaluated within the framework of what existing therapy can accomplish in each individual disease, and the potential for the new drug to enhance the curative potential of therapy. Our aim is not to find another agent that will be as good as 5-fluorouracil in large bowel cancer, but to find an agent that would be worth trying in combination with surgery, radiation therapy, immunotherapy and/or other drugs in the multidisciplinary therapeutic attack for cure that we feel is the future role for chemotherapy.

In line with this, the philosophy of phase III testing has also changed. The studies must be disease oriented and geared to the total flow of a patient's clinical history. An outline of such a flow for solid tumors in general is shown below:


SURGERY
Curative Resection "Good Risk"
Curative Resection "Poor Risk"
Palliative Resection
Inoperable
Observe or Treat
Treat Drug and/or X-ray vs Control
Treat Regimen A vs Regimen B or Regimen A vs Control
Treat Regimen A vs Regimen B
RECURRENT DISEASE

PHASE II OR PHASE III STUDIES

Approaches such as the preceding one are being planned by each tumor oriented task force or working party specifically tailored to meet the exigencies of each individual tumor. It is through the preceding approach, with emphasis on the need for a multidisciplined attack, that the Chemotherapy Program hopes to improve the cure rates in the decade to come.

HONORS AND AWARDS

1. Carter, S.K. Elected to Membership in the American Association for Cancer Research.

2. Carter, S.K. Elected to Membership in the New York Academy of Science.

3. Carter, S.K. Appointed Editorial Advisory Board, European Journal of Cancer.

4. Carter, S.K. Forsyth Memorial Cancer Lectureship, 1972.

PUBLICATIONS

1. Carter, S.K. and Carbone, P.P.: Current studies in hematologic malignancies. In Elkerbout, F., Thomas, P. and Awaveling, A. (Editors): Cancer Chemotherapy. Baltimore, Md., Leiden University Press, 1971, pp. 64-83.

2. Carter, S.K.: Clinical trials - combination chemotherapy. Cancer Chemother. Rep. (part 3) 2: 81-97, 1971.

3. Carter, S.K. and Friedman, M.: Dimethyl triazeno imidazole carboxamide - a new agent with activity in malignant melanoma. Europ. J. Cancer 8: 85-93, 1972.

4. Broder, L. and Carter, S.K.: Islet cell carcinoma: Clinical freatures and results of therapy with streptozotocin. Proc. Amer. Assoc. Cancer Res. 13: 96- , 1972.

5. Author: Broder, L. and Carter, S.K.: Meningeal Leukemia. New York, Plenum Press, 1972, 200 pp.

PRESENTATIONS

1. Carter, S.K., New Investigative Drugs Developed by the Chemotherapy Program of the National Cancer Institute presented at the Annual Cancer Symposium, St. Elizabeth Hospital, Youngstown, Ohio, April 17, 1971.

2. Carter, S.K., The Rationale of Screening Tests: A General

Introduction presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

3. Carter, S.K., Error and Compromise in Animal Screening presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

4. Carter, S.K., General Comments on Animals as a Tool for Prediction in Man presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

5. Carter, S.K., The Importance of Schedule in Clinical Trials presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

6. Carter, S.K., Some Thoughts on Phase II Trials Including the Evaluation of Tumor Response presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

7. Carter, S.K., Some Thoughts on Combination Chemotherapy of Breast Cancer presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

8. Carter, S.K., Some Thoughts on the Difficulty of the Interpretation of Response in Bronchogenic Carcinoma presented at the EORTC Clinical Pharmacology Course, Institut Jules Bordet, Brussels, May 10-13, 1971.

9. Carter, S.K., Status of Nitrosoureas, Bleomycin and other New Investigative Agents presented at the post-graduate course in Modern Clinical Cancer Chemotherapy, Mt. Sinai Hospital, New York City, May 28, 1971.

10. Carter, S.K., New Drug Information presented at the Symposium on Liver Cancer, Kampala, Uganda, July 5-7, 1971.

11. Carter, S.K., Clinical Studies with the Nitrosoureas: BCNU, CCNU and Streptozotocin presented at the Seventh International Congress of Chemotherapy, Prague, August 23-27, 1971.

12. Carter, S.K., Chairman, Afternoon clinical session of the International Symposium on Adriamycin, Milan, Italy, September 10-12, 1971.

13. Carter, S.K., Combination Chemotherapy in Breast Cancer presented at the National Advisory Cancer Council, October 5, 1971.

14. Carter, S.K., Hodgkin's Disease: Results with Single Agent Chemotherapy presented at the Cancer Clinical Investigation Review Committee, Symposium on Hodgkin's Disease, St. Louis, Missouri, October 7-9, 1971.

15. Carter, S.K., The Search for Therapeutic Cell Controls presented at the National Institute of Arthritis and Metabolic Diseases Workshop on Cell Controls in Psoriasis, Bethesda, Maryland, October 19-21, 1971.

16. Carter, S.K., Status of New Compounds presented at the Second Joint Working Conference, National Cancer Institute Chemotherapy Program, Annapolis, Maryland, November 3-5, 1971.

17. Carter, S.K., Some Thoughts on What Has Been Accomplished with Standard Agents in Acute Myelocytic Leukemia presented at the National Cancer Institute meeting on the Current Status and Future Plans for Acute Myelocytic Leukemia, Bethesda, Maryland, November 12, 1971.

18. Carter, S.K., The Design of Clinical Trials in Cancer Chemotherapy as Illustrated by the Nitrosoureas and the Other Drugs sponsored by the Chemotherapy Program, U.S. - Japan Joint Seminar on Anticancer Drug Evaluation, Tokyo, Japan, January 24-25, 1972.

19. Carter, S.K., Review of Single Agent Data presented at the Breast Cancer Task Force Treatment Subcommittee Meeting to Review Current Studies, Bethesda, Maryland, January 31, 1972.

20. Carter, S.K., New Drugs and Phase II Studies in Breast Cancer presented at the Breast Cancer Task Force Treatment Subcommittee Meeting to Review Current Studies, Bethesda, Maryland, January 31, 1972.

21. Carter, S.K., Methyl CCNU and Other Nitrosoureas presented at the Johns Hopkins Hospital Oncology Seminar, Baltimore, Maryland, February 8, 1972.

22. Carter, S.K., The Chemotherapy Program's Linear Array- Its Structure and Function. FDA - ASPET Seminar, Aerlie House, Virginia, March 6, 1972.

23. Carter, S.K., New Drugs on the Horizon in Bronchogenic Carcinoma presented at "Carcinoma of the Lung - A Status Report of the National Cancer Institutes' Current Research," Bethesda, Maryland, March 28, 1972.

24. Friedman, M.A., New Agents in Cancer Chemotherapy presented at the Orange County American Cancer Society Symposium, Orange County, California, December 4, 1971.

25. Broder, L.E., Pyrimethamine presented at the National Cancer Institute Meeting on the Current Status and Future Plans for Acute Myelocytic Leukemia, Bethesda, Maryland, November 12, 1971.

26. Slavik, M., Chromomycin A_3 presented at Phase I-II Liaison Meeting, Bethesda, Maryland, March 9, 1972.

CLINICAL TRIALS SURVEILLANCE SECTION

Clinical Trials Surveillance Section was established in December 1971 to provide technical information services and programs supporting Cancer Therapy Evaluation Branch monitorship of clinical trials of anticancer agents.

The section has been engaged in initial tasks which not only meet various immediate needs but also will contribute to defining and developing the data base required in long-term operations. Working closely with Literature Research Section, Program Analysis Branch, literature searches are in process or have been completed on topics such as the following:

1. Chemotherapy of chondrosarcoma
2. Chemotherapy of osteogenic sarcoma
3. Chemotherapy of pancreatic cancer
4. Natural history of colon cancer
5. Overall therapy of breast cancer
6. Surgical adjuvant studies in cancer of the lung, breast, colon, stomach, etc.

Such information, in addition to answering daily needs, will provide essential background data for establishing disease oriented file systems. These will include thorough searches of the open literature on diseases of prime importance plus unpublished data in the Cancer Therapy Evaluation Branch and reports of the cooperative clinical groups. The files will be categorized (e.g., natural history, surgery, radiotherapy, chemotherapy, etc.) and include appropriate subdivisions (e.g., drug categories, specific drugs, drug schedules, etc.).

After the development of workable files, the next phase will require maintaining the currency of the data by continuing literature surveys and regular input of results of ongoing clinical trials. In regard to the latter, a pilot effort is in progress to prepare a master "book" of acute myelocytic leukemia protocols in use by the cooperative groups. This compilation could be updated as new results are reported and might serve as a prototype of similar disease oriented records for quick reference in daily Cancer Therapy Evaluation Branch activities. At the same time, these manual records could provide input to pilot projects for automating storage and retrieval of data and information from clinical trials. The final stages of the information will require much more sophisticated means for gathering and manipulating data to pass completely from manual to ADP systems.

Clinical Trials Surveillance Section also provided service in documenting the minutes and proceedings of meetings such as the recently published "Conference on the Current Status and Future Plans for Acute Myelocytic Leukemia" and the proceedings of the

Phase I-Phase II Liaison Meeting (March 9, 1972) which will be prepared in the near future. The section plans to increase its efforts in this area, thus reducing the demands on the time of the Cancer Therapy Evaluation Branch clinical staff for preparing informational material of this type.

DRUG LIAISON AND DISTRIBUTION SECTION

The Drug Liaison and Distribution Section is responsible for compliance with Food and Drug Administration regulations regarding the use of drugs by the various clinical investigators, and for the distribution of drug to clinical investigators. In this section, the INDs are prepared and submitted to the FDA for an application, as required by law. The INDs are continuously amended and updated with the FDA upon receipt of protocols, reports, and any other pertinent information which is submitted to the Chief of the Cancer Therapy Evaluation Branch regarding the use of the drug in man. When requests for drugs are submitted, the drugs are routinely matched with their respective protocols. In this manner the section acts as a kind of monitor. An important function of the section is the distribution and maintenance of sufficient quantities of drug for investigators not only in this country, but abroad as well. This can be a difficult problem in that each investigational drug is beset with its own particular set of problems regarding the synthesis and formulation of bulk material. Supply problems may be further complicated when the drug enters Phase I clinical trial and the maximum tolerated dose in man is much higher than anticipated from the animal data.

The section works very closely with NCI staff who are involved in the procurement, synthesis, formulation, toxicology, pharmacology, and screening of drugs. In addition the Drug Liaison and Distribution Section works very closely with the various clinical investigators by communicating with them and by becoming familiar with their respective protocols. A formal mechanism of communication has been established with the Southwest Cancer Chemotherapy Study Group and the Central Oncology Group, which represent two of the largest cooperative cancer chemotherapy study groups in the country. In this manner the Drug Liaison and Distribution Section strives to coordinate the needs of the clinical investigator with the overall kinetics which lead to a sufficient quantity of drug for maximum patient benefit.

During the past year, six new drugs passed Decision Network 3 of the Chemotherapy Program Linear Array. These six drugs, for which the Cancer Therapy Evaluation Branch prepared and filed IND applications with the FDA, are as follows:

1. Cis-Platinum (II) Diamminedichloride (NSC 119875). This synthetic salt is one of the very few inorganic compounds presently undergoing clinical trials in man. The compound was first noted to have an antibiotic effect in 1965 and was since found to be an antitumor agent in experimental systems. These systems include the ascitic B16 melanosarcoma, Dunning ascitic leukemia, S180, W256, and L1210. In L1210, the compound shows activity over a variety of dosage schedules when administered IP. The maximum

ILS = 111% on a q3hx8, days 1, 5, 9 schedule. There is no activit when administered PO. There is a sensitive structure-activity relationship, as only the cis form of the salt is fully efficient for cell division inhibition. The trans form is relatively inefficient for the inhibition of cell division.

The compound is thought to exert its cytotoxic action through the selective inhibition of DNA synthesis at low concentrations, and inhibiting RNA synthesis and protein synthesis as well at higher concentrations. In both dogs and monkeys toxicology was manifest as GI in the form of enterocolitis and, at high doses, destruction of circulatory lymphocytes and hypoplasia of the bone marrow. Renal toxicity was indicated by tubular necrosis in dogs and interstitial nephritis in monkeys. The compound is currently undergoing widespread phase I trials at M.D. Anderson Hospital and the Sloan-Kettering Institute. The compound is formulated for parenteral administration as a 10 mg vial. To date, total doses have ranged from 45 mg-400 mg, and there is some evidence of activity in squamous cell carcinoma. Toxicities have included high tone auditory loss, renal damage and marrow suppression.

2. N-(2-chloroethyl)-N'(-2-chloroethyl)-N'-O-propylene phosphate diamide (Ifosfamide, NSC 109724). This synthetic compound, an alkylating agent, is an analogue of cyclophosphamide (Cytoxan, NSC 26271). This drug was initially investigated in Germany by the Asta-Werke Chemical Company, Brackwede, and has undergone clinical trials in that country. Compared to Cytoxan, ifosfamide demonstrates superior cytotoxic activity in the L1210 system. In addition, activity was noted in such experimental systems as the Lewis lung tumor, Erlich ascites, Rauscher and Yoshida sarcoma. In the L1210 system, the compound shows activity when administered IP and also when administered PO. The ILS is a >300% when administered IP once, on day 1, and also when administered PO once, on day 1. The optimal dose (OD), however, is higher for PO administration. German data indicate that a single IP dose of 120 mg/kg of ifosfamide cured 90% of rats with DS-carcinoma, a chemotherapy resistant tumor, whereas the Cytoxan cure was about 50%. They have also presented data which indicate that ifosfamide has less cumulative toxicity than Cytoxan. That ifosfamide shows anticancer activity is somewhat unusual in that Cytoxan analogues which have alkyl groups substituted on the ring nitrogen showed little ability to inhibit tumors in previous testing. As with Cytoxan, the compound shows no activity in vitro, and obtains its cytotoxic activity when metabolized. According to Canadian workers, ifosfamide acts in a synergistic manner with the nitrosourea BCNU (NSC 409962), but not with Cytoxan. Also, the use of ifosfamide as an immunosuppressant has been suggested. Toxicities which have been investigated by German investigators include nausea and vomiting, cystitis, leukopenia and alopecia. As with Cytoxan, thrombocytopenia is rare. Three different dosage regimens have been utilized in Germany, including IV and PO administration. Promising clinical results have been indicated in oat cell carcinoma of the lung, re-

ticulum cell carcinoma and Hodgkin's disease. The response rate with lymphosarcoma, however, appears inferior to Cytoxan. In this country, ifosfamide is currently undergoing phase I clinical trial at the VA Hospital-NCI, where the drug is being administered IV as a single dose every three weeks.

3. Rifamycin SV (Rifocin®, NSC 133100) is an antibiotic which was isolated in the Lepetit Research Laboratories, Milan, Italy. It is derived from the microorganism Streptomyces Mediterranei, and has had widespread clinical use for the treatment of bacterial, and especially tubercular infections. The discovery of RNA dependent DNA polymerase has been linked with a viral etiology theory of leukemia in man, and the observation that rifamycin SV is selective in inhibiting RNA dependent DNA polymerase which was derived from human ALL has suggested the clinical trial of this drug in humans with leukemia. Rifamycin SV itself appears to show little toxicity; however, it is readily oxidized to rifamycin S, which is toxic. For this reason rifamycin SV preparations contain ascorbic acid for the purpose of keeping rifamycin SV in the reduced state.

In dogs who were administered Rifocin® IM at 20 or 40 mg/kg/d x 6 months, hepatotoxicity was observed in the form of connective tissue proliferation and vacuolar degeneration. Also, the kidneys and spleens showed slight lesions.

In man, toxicity includes diarrhea, reactions at the site of IV injection, and increased bilirubin levels due to temporary saturation of the liver. The drug is currently undergoing phase I trial at the National Cancer Institute's Medicine Branch where it has been administered IV at a dose of 1500 mg/m^2 q 8 h x 15 doses.

4. Chromomycin A_3 (Toyomycin, NSC 58514). Chromomycin A_3 is an anticarcinomatic antibiotic substance which was isolated in the Takeda Chemical Industries, Ltd., Research Laboratories in Japan. The drug is isolated from the culture of streptomyces griesus No. 7. The drug has had extensive clinical trial in Japan in patients with a variety of malignant tumors including gastric cancer, pancreatic cancer, breast cancer, rectal cancer, pulmonary cancer and lymphosarcoma. The drug may be administered by IV and IA injection, and by intrathoracic, intraperitoneal and intratumoral infusion. In animal studies conducted in Japan, the drug exerted life-prolongation in rodents inoculated with the Yoshida sarcoma. In this country, studies against the P388 leukemia showed an ILS = 120% when treated every day, day 1-5, IP, at an optimal dose of 0.25 mg/kg/day. The drug is thought to exert its anticancer activity by inhibiting the maturity of cells which participate in RNA synthesis or protein synthesis. It does not appear to inhibit the utilization of adenine by DNA. In addition, when HeLa cells and cells of normal lung tissue were cultivated in a medium containing the drug, only the HeLa cells were effected; this implies a kind of selective cytotoxicity. Workers at the Takeda Research Labor-

atories have established the MTD and LD_{50} in mice to be 0.5 mg/kg and 1.85 mg/kg respectively, for IV administration; for IP administration these values are 1.0 mg/kg and 2.12 mg/kg. In rabbits who were administered 100 mcg/kg/d x 60 d, no histopathologic changes were found in the bone marrow or liver. In Japan, patient have been treated with the drug by IV administration in total dose ranging from 2 mg to 70 mg. Side effects include pain followed by induration due to extravascular injection, nausea, vomiting, anorexia and slight hepatotoxicity. Only moderate leukopenia has been observed, and it is reversible upon discontinuance of the drug. No definite relationship has been established between dose and leukopenia. Chromomycin A_3, thought to have a high affinity for lymphatic tissue, has shown promising results for the treatmen of malignant lymphoma, ovarian cancer, bronchogenic cancer, and stomach cancer. The drug will be undergoing phase I clinical trial by the Southeastern Cancer Cooperative Chemotherapy Group, the Central Oncology Group and the Mayo Clinic.

5. Thalicarpine (NSC 68075). Thalicarpine is an alkaloid which is derived from the roots of several plant species of Thalictrum, and also from Hernandia ovigera. This agent represents the first of a new group of dimeric isoquinoline alkaloids, one part of whic is an aporphine and the other a benzylisoquinoline. Thalicarpine demonstrated activity in the Walker 256 system, but in the L1210 system. On a divided schedule, day 1 only, the highest ILS = 131%. Further studies indicate that the drug lacks schedule dependency. Very little is known about the cytotoxic mechanism of action for this agent, and it can only be said that protein synthesis is inhibited, probably by the aporphine structure of the molecule. The drug appears to demonstrate two modes of toxicological action: 1) the potent hypotensive effect which is observed when the drug is administered by rapid IV infusion, and 2) the usual long term organ toxicities caused by antineoplastic agents. In rats, the LD_{50} is 500 mg/kg for a single two hour IV infusion. In dogs who were administered the drug intravenously, doses of 10 mg/kg induced hypotensive effects. Lethality was produced at doses above 41.6 mg/kg/d x 2-5 days. However, when the drug was administered over a two hour infusion, no pharmacologic effects were observed. Similar findings were observed in Rhesus monkeys. The lethality appears to be due to some effect of the drug on the cardiovascular respiratory system. Also, the animal toxicology studies indicate that the rate of drug injection and the concentration of drug are important factors to consider. The drug is entering phase I clinical trial at the NCI-VA Medical Oncology Service.

6. 9-β-D-arabinofuranosyladenine hydrate (Adenine arabinoside hydrate, Ara-A, NSC 404241). This synthetic compound is a purine nucleoside analogue which has demonstrated antitumor and antiviral activity in experimental systems. The activity of Ara-A is related to its activation by a phosphorylase kinase system and its inactivation by adenosine deaminase. When Ara-A is converted to the 5'-triphosphate (Ara-ATP), it acts as a non-competitive inhi-

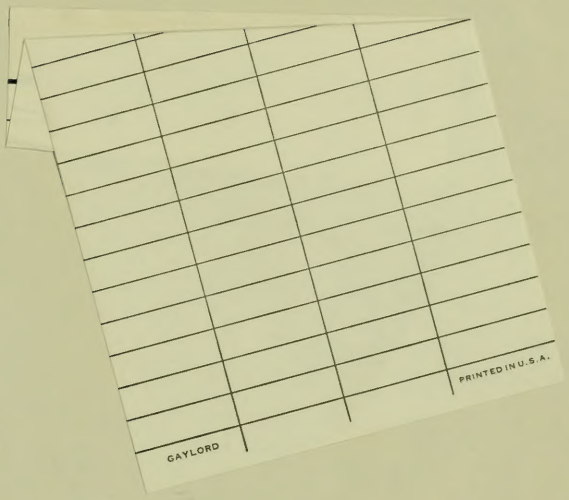
bitor of DNA polymerase and thus interferes with DNA synthesis. In experimental systems, Ara-A has shown antiviral activity against DNA viruses and against herpes simplex ophthalmia in the rabbit. Antitumor activity has been demonstrated against the ascitic tumors 6C3HED, Erlich, Adenoca 755 and TA3 which is also a breast tumor. No activity could be demonstrated against the L1210 system. Different degrees of tumor response can be explained in part by the ratio of the activiating enzyme (kinase) to the deactivating enzyme (deaminase). Some human tumors which have been studied in vitro have ratios of these two enzymes that are in the range of the ratios of the responsive animal tumors. This implies that the human tumors may also be subject to cytotoxicity by Ara-A. Animal toxicology has been studied extensively by Parke-Davis and Company, and signs of clinical toxicity to Ara-A were developed only by monkeys. The major toxicologic concern is central nervous system toxicity. Hepatotoxicity does not appear to be a problem at the doses studied, and there is very little or no bone marrow toxicity or GI toxicity. Indeed, Ara-A may exhibit a selective toxicity in that the human tumors studied have relatively low deaminase levels, whereas normal tissues have relatively high deaminase levels which inactivates the drug and protects from toxicity. In man, the drug has been administered IV for the treatment of herpes zoster at 10 mg/kg/d x 10 d with no toxicity. Ara-A is currently undergoing phase I clinical trial at M.D. Anderson Hospital.

PRESENTATIONS

1. Penta, John S., Some Problems Associated with Investigational New Drugs presented at the Central Oncology Group Meeting, Salt Lake City, Utah, January 14, 1972.

2. Penta, John S., Practical Aspects of Planning a 5-FU - Hydroxyurea Combination with Placebo for a Double-Blinded Study presented at the Chemotherapy Program Experimental Design Committee, NCI, Bethesda, Maryland, February 10, 1972.

3. Penta, John S., Status of Supply and Formulation Problems with Methotrexate presented at the Chemotherapy Program Osteosarcoma Meeting, NCI, Bethesda, Maryland, March 2, 1972.



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